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DEVELOPMENTAL STAGES IN THE DIGENEA. III. OBSERVATIONS ON THE NUMBER OF DAUGHTER SPOROCYSTS AND CERCARIAE PRODUCED IN *PHYSA GYRINA* AS A RESULT OF SINGLE AND MULTIPLE OCHETOSOMATID EGG-EXPOSURES

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INTRODUCTION

Recently Byrd and Scofield (1952) reported on the incidence of infection, rate of development, and hatchability of single and multiple ochetosomatid trematode eggs when ingested by a local snail (*Physa gyrina*). In the present paper data are presented relative to (1) the number of cercariae emerging from physid snails exposed to a single ochetosomatid egg; (2) the duration of the cercarial shedding period; (3) the number of daughter sporocysts produced by a mother and present when the snail host was sacrificed; (4) the number of cercariae emerging as related to the number of daughter sporocysts present; and (5) the pattern of cercarial emergence from the snail host.

MATERIALS AND METHODS

Snails of the genus *Physa* (*P. gyrina*) were used as the experimental host. These snails were raised in the laboratory and exposed to one or a number of ochetosomatid eggs according to the technic outlined by Byrd and Scofield (1952). The culture dishes (aquaria, stacking, 112 × 55 mm.) were kept partly filled with previously boiled spring water, and food and calcium carbonate were added as needed.

In the first series of experiments, snails shedding cercariae as a result of single egg-exposures were isolated throughout the remainder of their lives and for each snail data were obtained on the number of cercariae shed daily, the total number shed, and the length of the cercarial shedding period under laboratory conditions.

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In other experiments, snails shedding cercariae following exposure to one or a number of eggs were kept under observation long enough to determine the number of cercariae emerging over a short period of time before being sacrificed. The number of daughter sporocysts were counted in two ways: (1) the snails were killed by removing the shell, the body carefully dissected, and the daughter sporocysts counted, and (2) the snails were fixed entire in warm (60° C.) Bouin's fixative, embedded in paraffin, and sectioned at 8 microns before counting the daughter sporocysts. A slightly larger number was counted in the sectioned snails than in the dissected ones, with the difference being believed to be due entirely to the caution taken in converting broken pieces (resulting from the dissections) into entire sporocysts.

Emerging cercariae were counted at least once daily, usually between 9 A. M. and 12 Noon, as they were transferred individually to a second container. Frequently such counts were checked by a second person who transferred the cercariae individually to a third container. When two such counts differed, the cercariae were killed with formalin, transferred to glass slides after centrifugation, and counted under a compound microscope. In no instance did a daily count vary more than three cercariae for the three counts, an average of which (converted to the nearest whole number) being recorded as the count for that day.

In determining the cercarial emergence pattern, daily cercarial counts for those snails infected by a given species of fluke were arranged in tabular form, making it easy to determine (1) the number of days each snail shed cercariae; (2) the total number of cercariae shed by each snail; (3) the daily total shed by all snails; (4) the total number of cercariae shed by all snails; and (5) the total number of days in the cercarial shedding period. Once these numbers and totals were known, by trial a moving median was selected to illustrate the pattern. Consequently, separate moving medians were taken for 6-, 9-, 15-, and 21-day periods. Since medians for each of the interval periods were taken in the same way, the 9-day moving median should illustrate the method used. The daily totals for the first 9 days of the cercarial shedding period were summed and divided (1) by 9, and (2) by the total number of snails shedding cercariae each day of the period. By eliminating the total for the first day and adding the totals for the second through the tenth day, a median was obtained for the second 9-day period. Thus, by dropping the first and adding one additional day a moving median was taken for each of the 9-day periods. By dividing the totals for each period by the number of days in the period (9) and by the total number of snails shedding cercariae during the period, two sets of data were obtained, one showing the daily average for all snails and the other giving the average number of cercariae emerging per day from each snail (the last providing data on the number of cercariae per snail-day of activity).

Although no attempt was made to study infection rates in snails of different ages, the youngest snail used in the experiments was 89 days old and the oldest was 522 days of age, with the average age being about 230 days. All snails employed were raised from eggs in the laboratory.

RESULTS

The Number of Cercariae Emerging from "Positive" Snails. To determine the number of cercariae emerging from a snail following exposure to a single

ochetosomatid egg, each of 421 snails was exposed for a 24 hour period to a single egg of the ochetosomatid species listed in Table I. Forty-eight of the exposed snails ultimately shed cercariae, and all "positive" snails were individually isolated for the entire cercarial shedding period.

During the "positive" period a total of 39,759 cercariae was shed, with the cercarial shedding period ranging from 4 (*N. orula*) to 161 days (*O. ellipticus*), and the number of cercariae ranging from 2 (*N. orula*) to 2,816 (*O. ellipticus*) for a "positive" snail host.

In our experience, once cercariae emerged, the snail continued to shed them more or less regularly until the snail died, or until the infection became exhausted. It was in certain snails infected with *N. orula* and *D. conferta* that the main indication of "exhaustion" was obtained. In one instance (*N. orula*) an infected snail shed 2 cercariae on the first day, lived for three additional days, but failed to shed another cercaria. In another case a "positive" lived for 14 days and shed 5 cercariae (1 on the first, 2 on the fifth, 1 on the sixth, and 1 on the eighth day). Another snail lived for 35 days: it shed 449 cercariae, 441 of which were counted during the first 15 days, while 6 appeared during the next 5 days and the last 2 had emerged

TABLE I.—*The number of cercariae emerging from physid snails exposed to a single ochetosomatid egg*

Ochetosomatid species	No. snails exposed	No. snails shedding cercariae	Mean cercarial shedding period (range in days)	Mean no. cercariae counted per snail (range)	Total no. cercariae counted
Ochetosoma ellipticus	160	23	60.8 (6 to 161)	1151.5 (60 to 2816)	26,485
Neorenifer aniarum	32	9	26.3 (7 to 82)	1009.9 (20 to 2743)	9,089
Neorenifer orula	82	6	33.5 (4 to 68)	313.0 (2 to 619)	1,878
Dasymetra conferta	147	10	39.2 (12 to 60)	230.7 (12 to 707)	2,307
			Total all species		39,759

by the 33rd day. In another snail 404 of its 408 cercariae appeared during the first 47 days of the "positive" period. The snail lived for 21 additional days and shed the other 4 cercariae, one on each of 4 days, but shed none during the last 10 days of life. Of the 6 "positive" snails infected with *N. orula* only one shed cercariae every day (for a total of 35 days and 395 cercariae), while another specimen shed on every day except the last three (for a total of 45 days and 619 cercariae). Thus, these six snails shed cercariae on a total of 153 of the 201 days of "positive" life.

Only in one instance did a "positive" snail infected with *D. conferta* shed cercariae on each of the 12 days it lived, shedding a total of 123. Another snail lived for 14 days, but shed only 12 cercariae (6, 3, and 3 on the 1st, 5th, and 6th days, respectively). Another lived for 25 days and shed 26 cercariae (10, 14, 1, and 1, on the 1st, 2nd, 4th, and 18th days, respectively). A third snail lived for 52 days and shed a total of 16 cercariae, the last of which appeared on the 30th day of the "positive" period. Still another snail shed 108 cercariae over 56 days, with none appearing on 17 days of the "positive" period, and 10 of the cercariae-less days occurred immediately preceding death. Another snail shed 16 cercariae during the 52 days of its infected life as follows: 8 during the first 4 days and 8 between the 18th and 30th days of the infection. All 16 of the cercariae were shed on 11 days

while none appeared on 41 days, and 22 of the cercariae-less days immediately preceded death. All other snails of the series shed cercariae regularly, missing a day occasionally, or one to three days immediately prior to death. The 10 snails shed cercariae on 282 of the 399-day "positive" period.

Out of a total of 237 days in the "positive" period cercariae were shed on 233 days by the 9 snails infected with *N. aniarum*. Three of the 9 "positive" snails failed to shed cercariae on the day immediately preceding death and none emerged from one of these on the third day prior to death. The other six snails shed cercariae continuously throughout the "positive" period.

The 23 snails "positive" for *O. ellipticus* shed cercariae on 1,352 days of the "positive" period (1,398 days). Only 8 of the snails had a continuous cercarial shedding record. Six snails missed shedding cercariae only on one day, one of which missed the day immediately preceding death while the other five failed to release larvae on one day ranging from 4 to 24 days prior to death. The remaining snails in the series failed to shed cercariae on 2, 3, 5, 6, and 13 days, with 5 of them missing on the day immediately prior to death but with the other cercariae-less days being irregularly distributed over the "positive" period. One of these snails failed to shed cercariae for three days immediately preceding death.

One hundred seventy-eight was the largest number of cercariae (*N. aniarum*) to emerge from any one snail during a 24-hour period, with 159, 99, and 48 cercariae being the maximum for any day from snails infected with *O. ellipticus, N. orula,* and *D. conferta,* respectively.

The Pattern of Cercarial Emergence. As stated above, the moving median was employed in demonstrating the pattern of cercarial emergence. Since the curves resulting from the several series (6-, 9-, 15- and 21-days) of moving medians differed one from the other only in the degree of smoothness, only those curves based on the 9-day interval are presented (Graphs I–VI). Attention should be called to the fact that after all medians were taken, medians 1 and 2, 3 and 4, 5 and 6, etc., were averaged for the sake of shortening a graph. Thus, each bar in a graph represents an average of two moving medians.

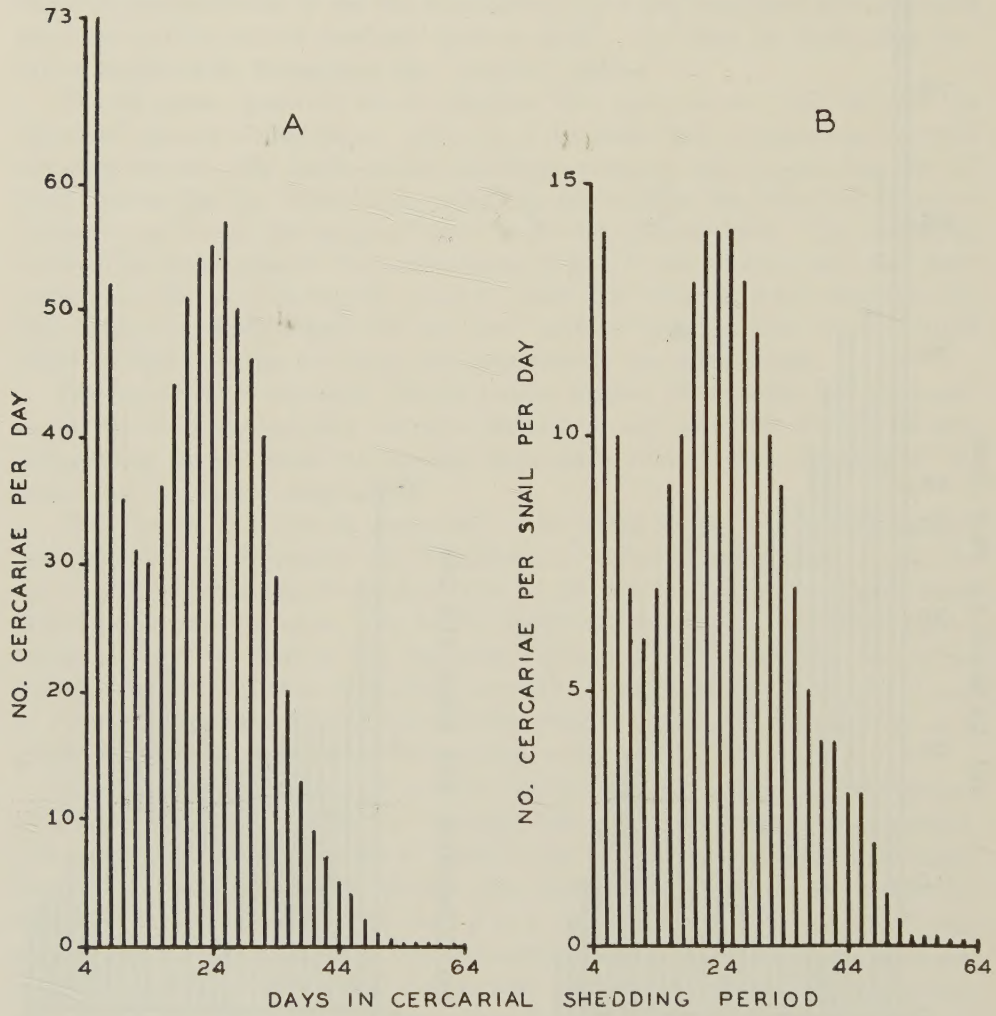
Regardless of the day-interval used in determining the medians, a basic pattern of cercarial emergence persisted for the other four species of ochetosomatids studied. The shedding period began with a "flushing-out" of larvae. Usually a moderately large number was released on the first day, followed by a more or less steady increase in the number during the next 2 or 3 days, culminating in an initial peak number by the 4th or 5th day. Moving medians based on a 9-day interval eliminated all evidence of this initial rise to peak numbers. Following the "flushing-out" period, the number of cercariae released daily declined through 10 to 20 days to establish a lower level of counts. From this level there was again an increase in the number, attaining a second peak by the 20th to 36th day following the first appearance of cercariae. In those cases (*N. orula* and *D. conferta*) where "positive" snails survived for no more than two months, the number of cercariae declined steadily from the second peak to the end of the experiment (death of snails). When "positive" snails survived (*N. aniarum* and *O. ellipticus*) longer than two months, the decline from the second peak was followed by at least one additional increase in the number of larvae emerging daily. From such a third peak, the daily number of cercariae declined to the end of the experiment.

In Graph I (snails infected with *D. conferta*) the daily output of cercariae never exceeded 8.3 cercariae per snail per day (Graph I, B) for the 10 infections. The decline in numbers from this initial high output was rather irregular but

GRAPH I (A and B). A bar-graph representing the daily emergence of cercariae from 10 snails when each snail host (*Physa gyrina*) was exposed to a single egg of *Dasymetra conferta*.

Note: Unless otherwise stated, this and all subsequent graphs present two curves for the daily emergence pattern of cercariae. The first curve (A) indicates the mean number of cercariae emerging daily from all snails in the series while the second curve (B) shows the mean number of cercariae emerging from each snail on each day of the infection. Both curves are based on a 9-day moving average and each bar in a graph represents an average for two of the 9-day periods.

reached a low of 4.8 cercariae per snail per day on about the 20th day. Although the mean total number of cercariae shed by each snail was small (230.7 cercariae per snail, Table I) it seemed reasonable to assume that a mean difference of 3 cercariae per day (Graph I, A) or of 1.7 cercariae per snail per day (Graph I, B) between this low level and the succeeding larger numbers might indicate that a second rise occurred in the daily output of larvae. If this succeeding higher level

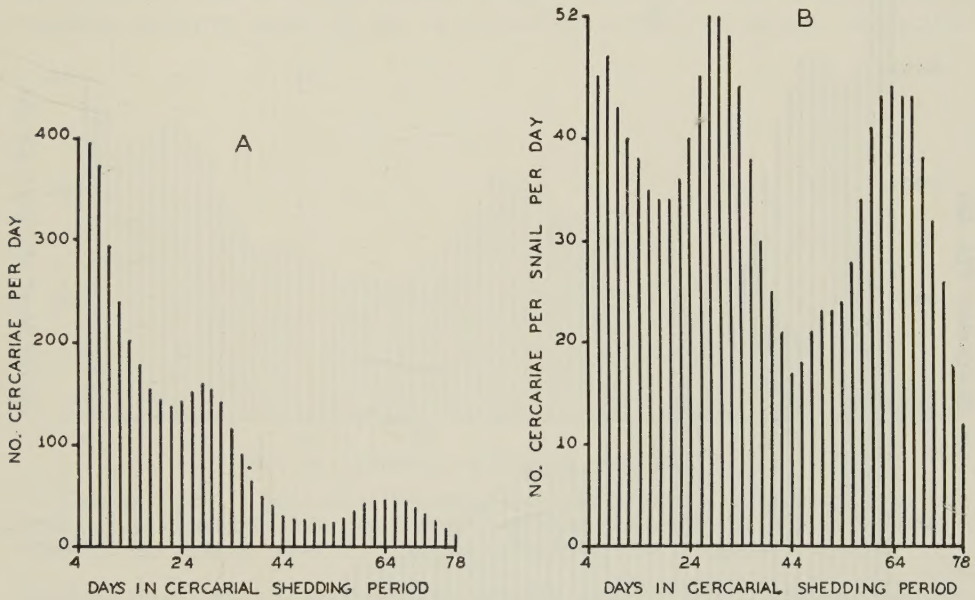


GRAPH II (A and B). A bar-graph representing the daily emergence of cercariae from 6 snails when each snail host (*Physa gyrina*) was exposed to a single egg of *Neorenifer orula*. See note for Graph I for further explanation of the graph.

can not be regarded as a significant rise, the increase must be considered as a recovery from the slump. Due, then, to the extremely small numbers of cercariae per snail per day even in the initial cercarial shedding period, the difference here of 1.7 cercariae per snail per day might be considered as significant for this species. Certainly the interval of 36 days elapsing between the first appearance of cercariae and the peak numbers during the second rise (or recovery period) is of interest

when compared with the data for the other species of ochetosomatids studied. Following the second peak (or recovery) the number of cercariae declined regularly until the end of the experiment, with no snail in the series living more than 60 days.

The data presented in Graph II (snails infected with *N. orula*) paralleled that given in Graph I. In this experiment, however, all snails were dead on or before the 68th day of cercarial shedding. From Graph II it was noted that (1) the decline in cercarial numbers following the initial flushing was extremely regular and sharp; (2) the lower level of counts was attained within 14 days; (3) the second rise attained a level as high as the initial peak numbers (Graph II, B); and (4) the interval between the two peaks was much shorter here (18 days) than was noted for *D. conferta* (Graph I, B). Further, the difference between the smaller counts and the succeeding peak was 8 cercariae per snail per day (Graph



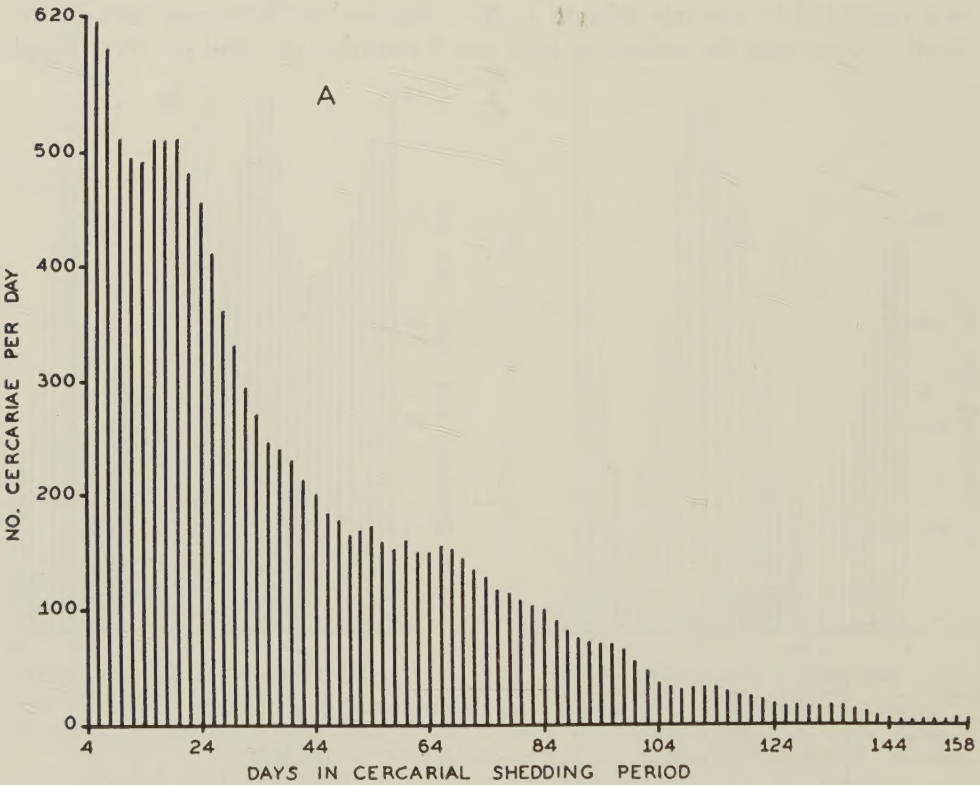
GRAPH III (A and B). A bar-graph representing the daily emergence of cercariae from 9 snails when each snail host (*Physa gyrina*) was exposed to a single egg of *Neorenifer aniarum*. See note for Graph I for further explanation of the graph.

II, B), or a mean of 27 cercariae per day for all snails (Graph II, A). The final decline in cercarial numbers, from the second rise to the end of the experiment, was much more gradual than was the case with *D. conferta*.

In Graph III (snails infected with *N. aniarum*) the emergence pattern paralleled that indicated in Graphs I and II, except for one additional "peak-cycle." The initial smaller count (34 cercariae per snail per day) was reached 14 days after the initial peak of 48 cercariae per snail per day (Graph III, B). Following the smaller counts, the number of cercariae increased to a peak by the 28th day, and the mean number of cercariae per snail per day (52) attained was at least 4 cercariae per snail per day greater than that of the initial peak. From the peak attained during the second rise there was a steady and rather precipitous decline in the numbers until a low of 17 cercariae per snail per day (Graph III, B) was reached, with the decline continuing over 14 days. Following this lower level

there was a more gradual (over 20 days) increase in the counts to a third peak of 45 cercariae per snail per day, just 7 cercariae per snail per day fewer than the largest number attained during the experiment. The third peak numbers persisted for about the same length of time as the previous two peaks, after which the mean numbers slumped to a low of 12 cercariae per snail per day at the end of the experiment. The decline in numbers following the second and third peaks was regular and precipitous. No snail in this series shed cercariae for more than 82 days.

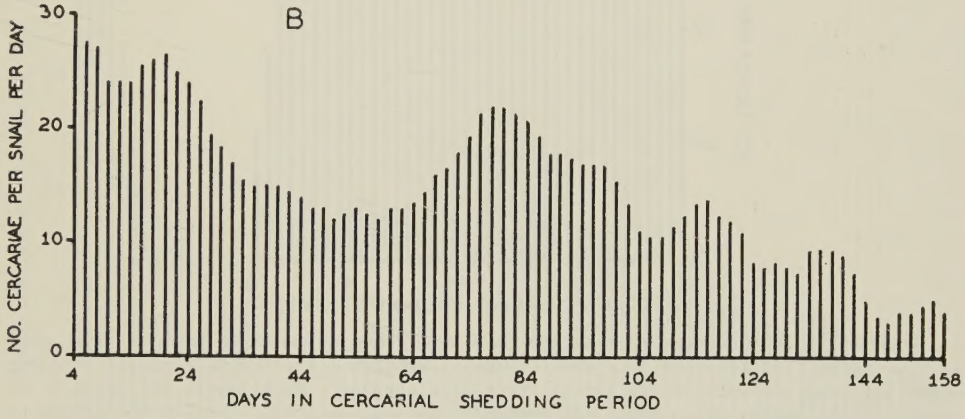
In the last experiment (Graph IV, snails infected with *O. ellipticus*), several apparent differences in the pattern of cercarial emergence were noted. The initial



GRAPH IV A. A bar-graph representing the mean daily number of cercariae emerging from 23 physid snails following exposure of each snail to a single egg of *Ochetosoma ellipticus*. See note for Graph I for further explanation of the graph.

flushing period was relatively short in duration, with the following lower level being only 3.5 cercariae per snail per day smaller than the initial peak numbers (Graph IV, B). The smaller numbers were reached within 10 days after the first appearance of cercariae. The subsequent increase in counts was less spectacular and attained a maximum of only 2.5 cercariae per snail per day greater than the preceding smaller counts. The second peak was attained within 14 days following the initial peak number. With these two peaks coming so close together, and with the intervening smaller numbers being of such an insignificant magnitude for this infection, it appeared here that only one peak might have occurred in the early phase of cercarial emergence, and that, with some fluctuation, this peak persisted

for a considerably longer period of time than was true for the previous three species. The rate of decline in cercariae following these larger counts was rather slow and irregular, with the succeeding smaller mean count (13 cercariae per snail per day) being taken 50 days after the first appearance of cercariae. The period of smaller counts persisted for several days, and then was followed by a steady increase until a new, "second" peak of 22 cercariae per snail per day was attained. This "second" peak was attained approximately 78 days after the first appearance of cercariae. From the "second" peak level the numbers of cercariae declined irregularly to the end of the experiment. As can be seen in Graph IV, B, this irregular decline was marked by what might be interpreted as a succession of peaks and slumps. If this interpretation can be considered as valid, then it must be noted that the several peaks occurred regularly at approximately 20-day intervals until all snails were dead, with at least four such regular peaks occurring. If the interpretation of regularly recurring peaks can not be considered as valid, the decline in cercarial

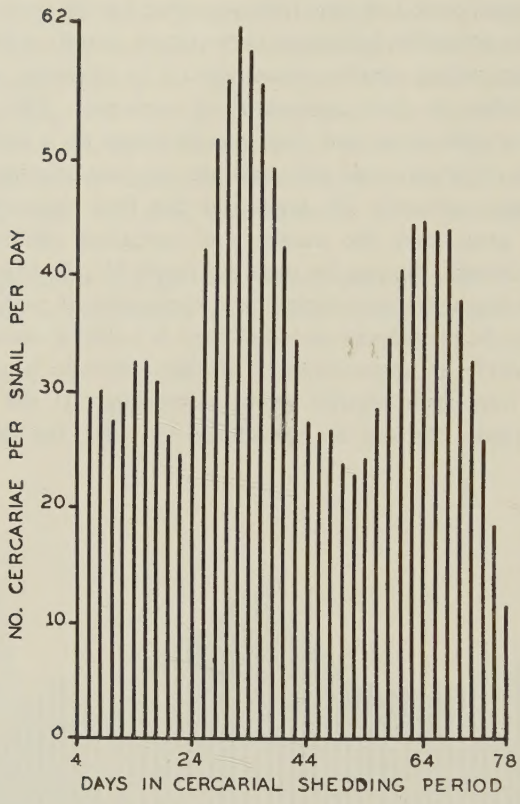


GRAPH IV B. A bar-graph representing the mean number of cercariae escaping daily from each of 23 physid snails following their exposure to a single egg each of *Ochetosoma ellipticus*. See note for Graph I for further explanation of the graph.

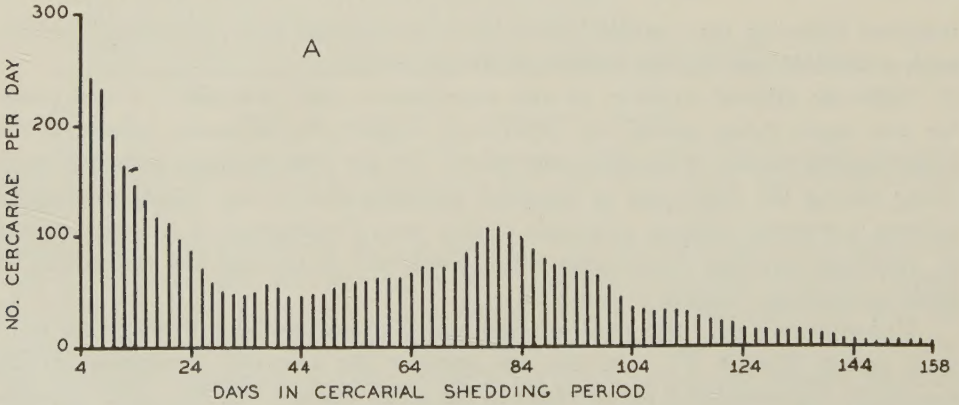
numbers following the "second" peak must be regarded as a "pulsating" decline, with a more or less regular rhythm at 20-day intervals.

Since the infected snails in all four experiments died "naturally", it was possible that snails dying during the experiment might have adversely influenced the demonstrated pattern of cercarial emergence. To see what probable influence snails dying during the early part of cercarial shedding had on the overall emergence pattern, a separate analysis was made of data on the emergence of *N. aniarum* and *O. ellipticus* cercariae from snails which lived into or through the last period of peak production (Graphs V and VI).

Unfortunately only one snail infected with *N. aniarum* lived through the third peak period (Graph V). In this one instance the cercarial emergence pattern essentially duplicated that for all snails in the series (Graph III, B). The principal differences in the two graphs are (1) the almost complete absence of a clear-cut initial peak in the single snail, resulting thereby in a more or less "plateau-like" level of cercariae in the initial phase, and (2) a slightly lower level between the second and third peaks.

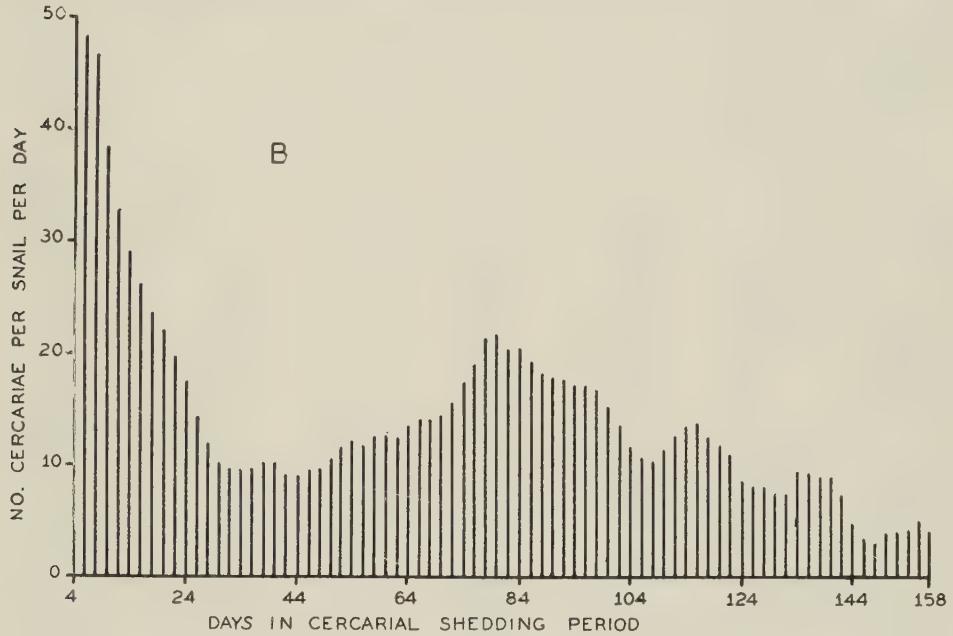


GRAPH V. A bar-graph showing the daily emergence of cercariae from the one snail (*Physa gyrina*) which lived and shed cercariae throughout the entire cercarial shedding period following exposure to one egg of *Neorenifer aniarum*. Each bar in the graph is an average of the number of cercariae emerging from the snail over two of the 9-day periods of the 9-day moving average. Graph V is to be contrasted with Graph III B, and the contrast readily illustrates what effect snails dying during the cercarial shedding period had on the overall pattern of cercarial emergence.



GRAPH VI A. A bar-graph showing the mean daily number of cercariae emerging from those five physid snails which lived and shed cercariae into or through the "second" peak numbers of larvae following the exposure of each snail to a single egg each of *Ochetosoma ellipticus*. When Graph VI A is contrasted with Figure A of Graph IV the effect produced by snails dying during the experiment is readily illustrated. See note for Graph I for further explanation of the graph.

Five of the snails infected with *O. ellipticus* lived into or beyond the "second" peak (Graph VI, A and B). A comparison of these curves with Graph IV, A and B, revealed a very close similarity, suggesting that the declines and increases in the numbers of cercariae during the course of the infection probably were inherent in the fluke species, and not due to the statistical analysis. The effects produced by snails dying during the experiment are clearly seen when the two sets of curves are compared. In making the comparison it should be remembered that the moving medians were determined by summing the daily totals of cercariae for all snails over a 9-day period and dividing by 9 (the number of days in each mean period)



GRAPH VI B. A bar-graph showing the mean number of cercariae emerging each day from each of the five physid snails which lived into or through the "second" peak numbers of larvae following the exposure of each snail to a single egg each of *Ochetosoma ellipticus*. When Graph VI B is contrasted with Figure B of Graph IV the effect produced by those snails dying during the experiment on the overall pattern of cercarial emergence is clearly seen. See note for Graph I for further explanation of the graph.

for Graphs IV, A, and VI, A, and the total number of snails shedding cercariae for each of the 9-day periods for Graphs IV, B, and VI, B. In either case the mean was plotted as occurring on the fifth day, making a bar in a graph represent the mean for a period which extended for four days on either side of the bar. Therefore, some of the cercariae emerging from a snail dying on a given day were counted in the totals for the succeeding eight days. In Graph IV (based on all snails in the series) the medians are represented by larger numbers of cercariae than are those of Graph VI (based on cercariae escaping from snails living throughout the period), and these larger numbers persisted so long as snails continued to die. Specifically, then, the effects produced by the death of snails were (1) the production of a "false" slump and rise in the numbers of cercariae shortly after the initial peak (Graph IV, A and B), and (2) the maintenance of the medians at a higher

level throughout the initial flushing period and during the subsequent decline, resulting in a more or less gradual and regular decline in cercariae to the end of the experiment (Graph IV, A). On the other hand, when only those snails which survived into or through the "second" peak were considered (Graph VI, A) no such regular and gradual decline in cercariae occurred from the initial high to the end of the experiment. Instead there was a clear-cut and rather precipitous decline from the initial high level (eliminating the "false" slump and rise in cercarial numbers immediately following the flushing) to a much smaller number, reached on about the 30th day of shedding. The lower level persisted, with some fluctuations, for roughly three weeks, after which there was a more or less gradual increase in the numbers to a "second" peak approximately 80 days after the first appearance of cercariae. It is evident, therefore, that snails dying prior to the

TABLE II.—*The number of daughter sporocysts produced (and the number of cercariae liberated) in physid snails exposed to a single ochetosomatid egg*

Snail No.	Source of egg	No. days cercariae were shed	Total no. cercariae shed	No. sporocysts counted	No. cercariae produced per sporocyst	No. cercariae produced per sporocyst per day
1		65	783	46	17.02	0.26
2	*Ochetosoma*	66	1242	37	33.57	0.51
3	*ellipticus*	69	814	44	18.50	0.27
4		73	2090	110	19.00	0.26
5		73	673	27	24.93	0.34
6		73	2339	45	51.98	0.71
Total		419	7941	309		
Av.		69.83	1323.50	51.50	25.70	0.37
1		1	5	97	0.05	0.05
2	*Ochetosoma*	2	15	77	0.19	0.10
3	*magnus*	5	317	123	2.68	0.54
4		5	385	47	8.19	1.64
Total		13	722	344		
Av.		3.25	180.50	86.00	2.09	0.64
1		2	12	31	0.39	0.20
2		2	32	49	0.65	0.33
3		3	18	72	0.25	0.08
4	*Neorenifer*	4	144	68	2.12	0.53
5	*aniarum*	4	152	36	4.22	1.06
6		4	45	56	0.80	0.20
7		19	460	44	10.45	0.55
8		19	327	66	4.95	0.26
9		23	817	45	18.16	0.79
Total		80	2007	467		
Av.		8.90	233.00	51.89	4.30	0.48

occurrence of the "second" peak served to mask the appearance of the "second" peak in Graph IV, A (compare Graphs IV, A and VI, A). In a similar manner the inclusion of cercariae which had emerged from snails dying earlier probably assisted in producing the irregularity in the curve shortly after the initial flushing period (Graph IV, B), and helped to maintain the mean numbers of cercariae per snail per day at a higher level during the decline and subsequent peak periods shown in Graph IV, B (compare Graph IV, B and Graph VI, B).

The Number of Daughter Sporocysts and Cercariae Resulting from Infections Produced by Single and Multiple Egg-Exposures. After exposing snails to single ochetosomatid eggs, some of the "positive" snails were sacrificed and the number of daughter sporocysts present determined. Thus far a total of 19 snails which shed cercariae following exposure to a single egg each has been studied (Table II).

The 6 snails infected with *O. ellipticus* (Table II) shed a total of 7,941 cercariae over a mean of 69.83 days. When killed and examined these snails contained 309

daughter sporocysts, or 51.5 sporocysts per snail. Each sporocyst (mean number) had liberated a mean of 25.7 cercariae, or 0.37 cercariae per mean day of the infection.

The 4 snails infected with *O. magnus* (Table II) produced a total of 722 cercariae over a mean of 3.25 days. A total of 344 daughter sporocysts was recovered from the snails, giving an average of 86.0 sporocysts per snail, and each sporocyst had produced an average of 2.09 cercariae, or a mean of 0.64 cercariae per day of the infection.

TABLE III.—*The number of daughter sporocysts produced (and the number of cercariae liberated) in physid snails exposed to more than one ochetosomatid egg*

Snail No.	Source of eggs	No. of eggs used	No. days cercariae were shed	Total no. cercariae shed	No. sporocysts counted	No. cercariae produced per sporocyst	No. cercariae produced per sporocyst per day
1			2	7	34	0.21	0.11
2			2	8	67	0.12	0.06
3			2	23	36	0.64	0.32
4		2	2	100	64	1.56	0.78
5			4	165	39	4.23	1.06
6			19	547	64	8.55	0.45
7			23	772	66	11.70	0.51
Total			54	1622	370		
Av.			7.71	231.71	52.86	4.38	0.57
1			2	35	52	0.67	0.34
2			2	59	40	1.48	0.74
3			2	47	98	0.48	0.24
4		3	2	17	52	0.33	0.17
5			19	669	62	10.79	0.57
6			23	376	49	7.67	0.33
7			27	1287	67	19.21	0.71
Total			77	2490	420		
Av.	*Neorenifer aniarum*		11.00	355.71	60.00	5.93	0.54
1			2	3	84	0.04	0.02
2			2	152	72	2.11	1.06
3			3	136	44	3.09	1.03
4		4	3	101	49	2.06	0.69
5			19	595	42	14.17	0.75
6			23	545	34	16.03	0.70
7			27	511	52	9.83	0.36
Total			79	2043	377		
Av.			11.29	291.86	53.86	5.42	0.48
1			2	182	76	2.39	1.20
2			2	63	46	1.37	0.69
3			2	105	119	0.88	0.44
4		5	4	65	67	0.97	0.24
5			19	489	97	5.04	0.27
6			23	917	68	13.49	0.59
7			27	823	89	9.25	0.34
Total			79	2644	562		
Av.			11.29	377.71	80.29	4.70	0.42

The 9 snails infected with *N. aniarum* (Table II) shed cercariae for an average of 8.9 days, liberating a total of 2,007 cercariae. A total of 467 sporocysts was counted. Each sporocyst, thus, could have produced an average of 4.3 cercariae for the mean cercarial shedding period, or each sporocyst accounted for approximately 0.48 cercariae per day of the infection.

Similarly data were available from 28 snails infected with the developmental stages of *N. aniarum* following exposure to more than one egg each (Table III). Of these 28 snails, 7 were infected following exposure to 2 eggs, 7 following exposure to 3 eggs, 7 following exposure to 4 eggs, and 7 shedding cercariae after exposure to 5 eggs.

In these experiments a known number of eggs was exposed to a single snail for a 24-hour period. Since all effort to recover unhatched eggs from the exposure

chambers (Syracuse crystals) failed to reveal the presence of eggs, there was no way of knowing just how many of the exposed eggs were picked up by a snail, nor could it be ascertained if the resultant infection in a snail represented the progeny from a single or from all eggs to which a snail was exposed. Regardless of the number of eggs actually represented by the data from a snail, Table III shows that (1) from 2-egg exposures each snail produced an average of 52.86 sporocysts and each sporocyst gave rise to an average of 0.57 cercariae per day of the infection; (2) when 3 eggs were exposed each "positive" snail harbored an average of 60 sporocysts, and each sporocyst produced an average of 0.54 cercariae per day of the infection; (3) the 4 egg-exposed snails averaged 53.86 sporocysts per snail, and 0.48 cercariae per sporocyst were liberated for each day of the infection; and (4) 0.42 cercariae per sporocyst per day of the infection were liberated by each of the 80.29 sporocysts per snail following an exposure of 5 eggs to each snail.

Coefficient of Correlation. In working up the data relative to the sporocysts-cercariae production from single and multiple egg-exposed snails the coefficient of correlation was determined for (1) the length of the cercarial shedding period and the number of sporocysts present; (2) the length of the cercarial shedding

TABLE IV.—*The coefficient of correlation between length of cercarial shedding period, number of cercariae, and sporocysts produced by snails exposed to single and multiple numbers of ochetosomatid eggs*

No. eggs exposed	Length of cercarial shedding period and no. sporocysts present	Length of cercarial shedding period and no. cercariae counted	No. sporocysts present and no. cercariae counted
1	−.0101	.9249	−.1442
2	.5108	.9875	.5153
3	−.0169	.8847	.1182
4	−.5686	.9373	−.6743
5	.1362	.9585	.0829

period and the number of cercariae emerging; and (3) the number of sporocysts present and the number of cercariae released. Since 9 of the snails shedding cercariae following exposure to a single egg and all of the snails exposed to a multiple number of eggs were exposed to eggs from the same species of ochetosomatid (*N. aniarum*), only the data from this series of snails were used in the calculations. The coefficient of correlation determined for each of the three categories, and based on snails exposed to 1, 2, 3, 4, and 5 eggs, are shown in Table IV.

From the information at hand all five groups of the "positive" snails gave a positive coefficient of correlation for the length of the cercarial shedding period and the number of cercariae released. For this relationship an unusually high correlation was demonstrable. This high positive correlation, as one would suspect, indicated that the number of cercariae produced during an infection bears a direct relationship to the length of time the infection continues in the host. The number of sporocysts produced and the number of cercariae released by each sporocyst depend on factors other than the duration of the infection. Admittedly, the number of infected snails employed in these experiments was so small that any calculated indication of a positive correlation is unreliable. Nevertheless, time in term of days an infection may continue certainly would favor the production of

an increasing total number of cercariae regardless of the number of sporocysts (or rediae) involved in their production.

DISCUSSION

From experiments in which each *Pseudosuccinea columella* was exposed to a single miracidium of *Fasciola hepatica,* Krull (1941) reported the number of cercariae to emerge ranged from 14 to 629 per snail host. On the other hand, the observations made by other workers (Ameel *et. al.,* 1949, 1950a, b, 1953; Cort, 1922, 1944; Cort *et. al.,* 1941, 1943a, b, 1944, 1944, 1948, 1949, 1950a, b, 1952, 1953; Faust and Hoffman, 1934; Olivier and Mao, 1949; Rothschild, 1939, 1942; Schreiber and Schubert, 1949a, b; and others) have indicated that a much larger number of cercariae might be expected, with some workers indicating as many as a million might emerge from a single snail host.

Since no effort was made to follow the development of the germ cells of the miracidium through the mother and daughter sporocysts to the production of cercariae, there was no way of correlating the final number of cercariae counted from each snail in these experiments with the germ cell-germinal mass reproductive potential indicated for other plagiorchiid trematodes. Although Cort, Ameel, and Van der Woude (1952) made no estimate of the possible number of cercariae which might result from a single miracidium of *Lechriorchis primus* Stafford, 1905 (a plagiorchiid species which is more closely related taxonomically to the 4 species studied in the present paper than are the other plagiorchiids investigated by Cort *et. al.*), they inferred a rather large number might be expected. In a paper by Cort and Ameel (1944), in which several species of plagiorchiids were studied, it was stated (p. 52): "The combination of the extensive multiplication of the cells of the germinal line in the mother sporocysts which is interpreted as a primary polyembryony of the zygote (fertilized ovum) with the secondary polyembryony of the germ-masses of the daughters, produces the enormus numbers of cercariae so characteristic of the plagiorchiids. In a species like *A. corti* where the number of daughter sporocysts is unusually great, and each daughter is large, it seems possible that more than a million cercariae can be produced from one fertilized ovum." It, thus, seems apparent that from a study of the germ cell-germinal mass type of embryo production in the plagiorchiids one could expect unusually large numbers of cercariae to result from a single fertilized ovum. The actual numbers of cercariae obtained from the ochetosomatids tested, however, were in the tens and hundreds rather than in the thousands and millions.

The data from several groups of "positive" snails (Tables II and III) indicated that a larger number of daughter sporocysts might be expected from younger infections than from older ones, and that a slightly larger mean number of cercariae might be produced daily by younger daughters. This suggestion was particularly evident from the series of snails infected with *O. magnus* (Table II), in which each snail harbored a mean of 86 daughters (from 47 to 123) and in which each daughter produced a mean of 0.64 cercariae per day (3.25 days). In this case, however, the larger number of daughters per snail and the larger number of cercariae per sporocyst per day might just as well be attributed to a greater reproductive potency of the species as to the youthfulness of the infection. The youthfulness of the infection appeared to be the less likely of the two explanations

since in *N. aniarum* (Table II) the first 6 snails listed in that series shed cercariae a comparable length of time (3.16 days) but failed to yield any more sporocysts or cercariae than did all snails in the group.

It was hoped that the number of daughter sporocysts present and the number of cercariae emerging from snails in the short-time experiments would provide information useful in determining the number of mother sporocysts actually involved in the production of daughters. No such information was obtained. The mean difference between the number of daughters per host for the single and multiple egg-exposed snails probably failed to indicate that one group acquired an additional mother. It is possible, however, that in the absence of competition a single mother sporocyst could produce a number of daughters and cercariae equivalent to that produced by two or more mothers developing in competition with each other. Regardless of the number of mother sporocysts involved in their production, these experiments failed to indicate that the number of cercariae produced per sporocyst per day was greatly influenced by the number of daughters harbored by a snail.

Although very little experimental evidence was obtained to substantiate the claim, the short survival time of snails harboring the developmental stages of *D. conferta* and *N. orula* might suggest that the local physid is less suitable as a host for these two species than for *O. ellipticus* and *N. aniarum.* As was indicated by Table I and Graphs I and II, no snail infected by *D. conferta* lived longer than 60 days after the first cercariae appeared, or beyond 68 days when infected with *N. orula.* The physid did allow for the development of a slightly larger mean number of *N. orula* cercariae per experimental host than for *D. conferta.* It could be that *N. orula* was the more vigorous of the two species and, hence, capable of producing more cercariae. On the other hand, the much smaller percentage of infection in the exposed snails and the smaller number of cercariae released over approximately the same survival period (Table I) for those snails infected with *D. conferta* might be substantiation for the contention of Byrd and Scofield (1952) when they stated (p. 535) "It is suggested, therefore, that the local physid, although probably serving as the natural local host, is less susceptible to this fluke than is true for the other ochetosomatids tested." Accordingly, the information presented herein might suggest that the local physid was more acceptable as a host for *O. ellipticus* and *N. aniarum,* or that these two species were capable of producing much larger numbers of cercariae. With these two flukes the cercarial shedding periods extended over 161 and 82 days, respectively (Table I and Graphs III and IV).

Regardless of the longevity of the infection or the assumed compatibility or incompatibility of the parasite and host, an overall general pattern of cercarial emergence from the snail hosts prevailed, and was quite similar, for the four species, especially during the early phase of the shedding. The larger numbers of cercariae appearing during the first few days probably indicated an accumulation of larvae in the tissue of the snail during the unknown period between their release from the daughter sporocysts and their emergence from the host, or a greater reproductive potential in young, mature daughters.

In consideration of the first of these possibilities, it is known that some species of flukes release cercariae from sporocysts (or rediae) in an "immature" stage

while in others the cercariae are released in a "mature" state. When released in an "immature" stage, the larvae complete their development in the tissues of the host before emerging, whereas if they are "mature" before leaving the sporocyst (or redia), the interval between their release and their emergence should be short, although varying somewhat from species to species. Undoubtedly, when cercariae are released in an "immature" stage the releasing process is a continuous one, and as the tissue-maturing ones emerge from the snail other "immature" larvae move out from the sporocyst (or redia) to replace them. Thus, the actual pattern of emergence for these cercariae should not differ from that displayed by larvae leaving the sporocyst (or redia) in a "mature" condition. It would seem, therefore, that an accumulation of cercariae in the tissues of the host is inadequate to explain the initial flushing-out of cercariae experienced in these studies. Further, if an accumulation of cercariae in the hosts' tissues could explain the initial flushing of larvae, it would have to be assumed that several such accumulations occurred during the larval shedding period in those snails harboring *N. aniarum* and *O. ellipticus* (Graphs III and IV).

One further idea relative to the accumulation of cercariae in the tissues of the host may be examined. For the 48 snails which ultimately shed cercariae following exposure to one egg (Table I) the first cercariae appeared after a mean of 34.1 days. The means of the minimal (snails shedding cercariae within a shorter time than 34.1 days) and maximal (snails having this period longer than 34.1 days) number of days were 28.9 and 45.7, respectively. If the minimal and maximal means can be used as standards for the variation around the mean for all snails in the series, it can be pointed out that 33, or 68.75 per cent, of the 48 hosts shed larvae after a mean of 5.2 days less than the mean for all snails while 15, or 31.25 per cent, required 11.6 days more than the mean for all snails. Thus, if an accumulation of larvae had occurred, a larger number of larvae might have been expected during the initial phase of shedding from those snails requiring the longer mean period. No such larger numbers of cercariae occurred. Neither was it possible to detect any correlation between the length of cercarial development and the total number of cercariae emerging, nor for the length of cercarial development and the duration of cercarial shedding.

As to the reproductive potential of the daughters (sporocysts or rediae) attention may be called to the incompleteness of the information available on the methods of reproduction and the reproductive capabilities of these stages. Many of the more recent investigators (Ameel *et al., loc cit.;* Brooks, 1930; Cable, 1934; Chen, 1937; Cort, *loc. cit.;* Cort *et al., loc. cit.;* Rees, 1940; Sewell, 1922; Willey *et al.,* 1941, 1947, 1950, 1951; Woodhead, 1931, 1950, 1952; van der Woude *et al.,* 1953; and others) have presented evidence suggesting the probable methods of reproduction in sporocyst-redia stages although there is no common agreement as to the exact details of the mechanism involved. Regardless of the mechanism involved, the type of development outlined by these workers suggests that the process is a continuous, uninterrupted one. One indication to the contrary appeared in a paper by Cort, Ameel, and van der Woude (1952) in which the sporocysts (2 generations) of a species, *Lechriorchis primus,* presumably belonging to the same subfamily (Ochetosomatinae) as the species dealt with in the present paper, appeared to show differences in the rate of their development.

The authors stated (p. 196): "It appears that cercarial embryos are first detached from the germinal mass at the beginning of the growth stages of the daughter sporocysts. As in other plagiorchioids, all later production of cercarial embryos appears to be by the germinal mass, which persists throughout the reproductive life of the daughters. The first mature cercariae that are ready to escape from daughter sporocysts as early as the 20th day after infection must therefore come from the early divisions of the germinal cells. There is also a considerable lag in the development of part of the daughter sporocysts in an infection. Mature cercariae may be present in some daughter sporocysts, while others are in the growth or even pre-growth stages. This lag is also shown in younger mother sporocysts in which germinal cells may still be present when some of the daughter sporocyst embryos are of considerable size. In mature and old infections that have evidently lasted over the winter all the sporocysts contain mature cercariae."

The significance of the "lag" in the development of some of the "mother" and "daughter" sporocysts is not fully understood. If by the "lag" it is meant that some of the sporocysts fail to reach maturity until others (the first ones) have passed their peak of cercarial production, then it might be argued that essentially two or more batches of daughters might result from a single continuous germinal line. Such a "rhythm" of sporocyst production in part might result in the occurrence of more than one peak in the number of cercariae emerging from an infected snail host. Such an explanation for the succession of peaks as shown in Graphs I–VI of the present paper, however, should assume a succession of "lags" in sporocyst development. Each "lag" period, thus, should have been sufficiently long for the first (or any) batch of daughters to have become senescent before the succeeding batch developed to productivity if the rhythmical recurrence of peaks in cercarial emergence (Graphs III, IV, V, and VI) was to be produced. Certainly it does not seem reasonable to explain the succession of peaks and slumps in cercarial emergence to a "lag" in the development of mother sporocysts, since here presumably only one such mother was present.

The demonstrated pattern of cercarial emergence for the larvae of the ochetosomatids studied, the number of daughter sporocysts resulting from one or more egg exposures and the duration of the cercarial shedding period suggest a need for further research on the details of the molluscan phase of the life cycle. The available information relative to these details provides very little knowledge on such important points as (1) just how long a sporocyst (mother and daughter) or redia lives with within the snail host; (2) the length of the reproductive period in the total life span of these stages; (3) the number of offspring each stage is capable of producing; (4) what percentage of those individuals "born" live to, through, or beyond the reproductive period; (5) the method of escape of cercariae from the snail host; (6) the intrinsic and extrinsic factors influencing cercarial emergence; (7) the nature of the snail's defensive response to the infection; (8) the influence of such a defensive response on the longevity and reproductive potential of the sporocyst or redia; and (9) the survival index of cercariae prior to their emergence. Admittedly the full elucidation of these unknowns will be accomplished with great difficulty.

From the data accumulated during the present study no satisfactory and constant correlation was obtained for the various factors analyzed except for a

positive relationship between the duration of the cercarial shedding period and the number of cercariae emerging from the snail host. For the species tested this relationship had a positive coefficient of correlation which ranged from 0.88 to 0.99. With knowledge of hindsight, it seemed evident that such a positive correlation should have existed, or that the longer an infection persisted the larger would be the number of cercariae to emerge. At the beginning of these experiments it was hoped that a constant and positive correlation could be demonstrated for the number of cercariae emerging and the number of daughter sporocysts in a snail host. No such positive correlation was found. The coefficient of correlation for this factor ranged from -0.67 to $+0.51$, a relationship which did not differ greatly from the -0.56 to $+0.51$ correlation for the duration of the emergence period (days) and the number of sporocysts counted. It does appear significant that the number of cercariae emerging from a host failed to give some direct indication of the number of daughter sporocysts producing them, and that the number of daughter sporocysts harbored by a snail should fail to indicate the number of mother sporocysts acquired by that host. In these experiments, however, attention should be called again to the fact that the "number of eggs used" was tne number of eggs to which a snail was exposed. All efforts failed to demonstrate whether or not a snail actually acquired more than one egg even though as many as 5 eggs were exposed. From the number of infections resulting from the various exposures it seems reasonable to assume that some of the snails were successful in finding more than one egg and, hence, their infection resulted from the establishment of more than one mother sporocyst. The negative correlation between number of eggs involved and the number of resultant daughter sporocysts was based on the above assumption. If the assumed could be taken as the actual, one might consider there was a corresponding reduction in the number of daughter sporocysts produced as the number of mother sporocysts was increased. This assumption must await experimental demonstration.

SUMMARY

In experiments dealing with *O. ellipticus, N. aniarum, N. orula* and *D. conferta* some information was obtained on the number of daughter sporocysts and cercariae produced when physid snails, *Physa gyrina,* were exposed individually to one or more eggs.

The number of cercariae shed by snails exposed to one egg of *D. conferta* ranged from 12 to 707, from 2 to 619 for snails harboring *N. orula,* from 20 to 2,743 for infections with *N. aniarum,* and from 60 to 2,816 for snails infected with *O. ellipticus.* In these tests each "positive" snail was kept isolated throughout the entire cercarial shedding period.

Daily counts were made on cercariae emerging from each snail over the entire cercarial shedding period, and no "positive" snail was considered to be free of the infection once cercariae emerged. All "positive" snails died "naturally" in the laboratory, with the cercarial shedding period extending over a mean of 60.8 days in the case of *O. ellipticus,* 39.2 days for *D. conferta,* 33.5 days for *N. orula,* and 26.3 days for *N. aniarum.*

The pattern of emergence was determined for the cercariae shed by snails exposed to a single egg each of *N. aniarum, N. orula, O. ellipticus,* and *D. conferta.*

Essentially the same pattern of emergence prevailed regardless of the fluke species employed, and this pattern was characterized by an initial "flushing-out" of cercariae, followed by more or less rhythmical decreases and increases in the daily numbers of cercariae throughout the remainder of the cercarial shedding period.

From short-time infections, in which the number of cercariae emerging from snails exposed to one egg each were counted prior to sacrificing the hosts, it was observed that each mother sporocyst of (1) *O. ellipticus* produced an average of 51.5 (27 to 110) daughter sporocysts, and each of these gave rise to 0.37 cercariae per day of the experiment; (2) *O. magnus* accounted for an average of 86 (47 to 123) daughter sporocysts per mother, with each producing 0.64 cercariae per day of the infection; and (3) each mother sporocyst of *N. aniarum* gave rise to an average of 51.9 (31 to 72) daughters, and each of these produced an average of 0.48 cercariae per day.

When snails were exposed to more than a single egg each (all eggs from *N. aniarum*) no significant difference was noted for the number of daughter sporocysts and cercariae produced as opposed to single egg-exposures. However, in these infections it was impossible to determine whether or not a snail exposed to 2, 3, 4, or 5 eggs actually acquired more than one mother sporocyst.

A constant and positive coefficient of correlation was demonstrated for the relationship between the duration of the infection and the number of cercariae emerging from the snail host. All other relationships studied varied from positive to negative for the several groups of snails.

In a discussion on the daily variations in the numbers of cercariae emerging from the snail hosts it was concluded that the phenomenon might just as well be due to an inherent character of the species as to intrinsic or extrinsic factors in the parasite-host relationship.

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PARADILEPIS RUGOVAGINOSUS N. SP. (CESTODA: DILEPIDIDAE) FROM THE OSPREY, WITH NOTES ON THE GENUS *OLIGORCHIS* FUHRMANN, 1906*

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An osprey, *Pandion haliaetus carolinensis* (Gmelin) was collected in May, 1952 and shipped to the Ontario Department of Lands and Forests Research Station at Maple, Ontario where it was frozen. The bird was examined September 26, 1952 and found to harbor five species of helminths, including several specimens of a cestode described here as *Paradilepis rugovaginosus* n. sp.

From a comparison of the several fragments present it is believed that at least four tapeworms were harbored by the host, although only two scoleces were recovered. The worms were fixed in F.A.A. without flattening. Two whole mounts, one stained with Mayer's Carmalum, the other with Mayer's Hemalum, were made. Frontal, transverse, and sagittal serial sections were cut at 10 microns and stained in haematoxylin and eosin, or in haematoxylin, eosin, and azure II. All measurements were made from stained and mounted worms except the maximum measurements of the scolex which were made from a fixed worm in wet mount.

Paradilepis rugovaginosus n. sp.

Description: Dilepididae Railliet and Henry, 1909; Dilepidinae Fuhrmann, 1907. Strobila at least 75 mm. long, delicate; greatest width 0.53 to 0.60 mm. near terminal segments. External segmentation prominent, strobila serrate. Segments ellipsoidal to subspherical in transverse section. Scolex 0.31 to 0.47 mm. wide, more than twice as wide as neck. Maximum width of suckers from 0.13 to 0.18 mm. Rostellum 0.14 mm. long by 0.13 mm. wide, armed with 32 hooks in two rows. Large hooks 0.099 to 0.103 mm. and small hooks 0.070 to 0.072 mm. in length (Fig. 3). Width of neck 0.10 to 0.20 mm. in narrowest region shortly posterior to scolex; remainder of strobila gradually widens posteriorly. Segmentation begins 1.4 mm. posterior to scolex; strobila with approximately 500 proglottids. All segments wider than long, but ratio of length to width is greater in the mature proglottids which measure 0.3 mm. long by 0.5 mm. wide. Musculature consists of a layer of thick longitudinal muscles two or three bundles deep (Fig. 4); transverse muscle fibers not observed. Longitudinal osmoregulatory canals 0.02 to 0.05 mm. in diameter; transverse canal variable, up to 0.012 mm. in diameter. An incomplete tube-like structure can be seen in serial transverse sections in position normally occupied by dorsal canal; however, the tubule disappears in vicinity of genital ducts, and it was not determined if genital ducts cross the osmoregulatory canals or pass between them. Primordia of cirrus pouch, vagina, and testes first noticeable 15 mm. posterior from scolex (ca. segment 230); other primordia not yet apparent. Genital pores 0.03 mm. in diameter, unilateral, sinistral, anterior to middle on proglottid margin; genital atrium 0.04 mm. deep. Typically four testes per proglottid, rarely three or five; testes varying in shape from oval to more elongate, 0.06 to 0.09 mm. long by 0.03 to 0.05 mm. wide. Testes mainly dorsal to plane of female organs, usually three more or less in a row along posterior edge and one more anterior near the aporal edge of the female genital complex (Fig. 2). Cirrus pouch, thick-walled, 0.24 to 0.30 mm. long and 0.08 to 0.10 mm. in maximum width, extends from genital atrium slightly anteriorly and inwardly beyond middle of segment. Cirrus, 0.23 mm. long and 0.03 mm. in maximum width, has well-developed stout spines. Spines vary from 5 to 13 microns in length

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by 1 to 4 microns in diameter at base; smaller spines at distal and proximal ends, with those of maximum size along middle two thirds of cirrus. Proximal portion of cirrus sinuous, unites with vas deferens which has a short convoluted portion within proximal part of cirrus sac; remainder of vas deferens prominent, long, and highly convoluted, and sometimes overlaps transverse and aporal osmoregulatory canals. Sperm ducts near dorsal margin of medullary parenchyma; ducts from each pair of lateral testes unite first and then these two ducts unite to form proximal end of vas deferens. Vas deferens not dilated into external or internal seminal vesicles. Vagina, which either everts or prolapses readily, prominent and ventral to cirrus pouch. Before reaching middle of segment the vagina bends sharply to the posterior and then dilates into a thick-walled seminal receptacle. Vagina is a tube within a tube (Fig. 5). The inner tube is strongly rugate and contains cilia-like structures arranged in circular bands, the latter corresponding to the rugae; the cilia, up to 27 microns in length, extend distally from their attachment on the vaginal wall. Vagina, unextended 0.14 to 0.20 mm. in length by 0.04 to 0.06 mm. in maximum width, when extended 0.22 to 0.29 mm. in length by 0.05 to 0.06 mm. in maximum width. Seminal receptacle 0.06 to 0.07 mm. in length by 0.04 to 0.05 mm. in maximum width. Ovary, up to 0.22 mm. in maximum width, situated posterior to seminal receptacle and ventro-anterior to testes, usually with four or five clavate lobes. Lobes in mature segments become sac-like with a thin isthmus connecting the laterally placed lobes (Fig. 2). Posterior and dorsal to median stem of ovary is the dark-staining yolk gland, 0.04 to 0.06 mm. in diameter, with the lighter-staining Mehlis' gland in close proximity but ventral to the latter. The uterus begins as two lateral out-pockets ventral to ovary; fully gravid segments were not observed.

Host: Pandion haliaetus carolinensis (Gmelin), the osprey.

Habitat: Small intestine.

Locality: Sault Ste. Marie, Ontario, Canada.

Types: Two whole-mount paratypes and two sets of serial sections have been deposited under No. 48788 in the U. S. National Museum Helminthological Collection.

DISCUSSION

Paradilepis rugovaginosus n. sp. is the third species of cestode to be recorded from the osprey. Previously von Linstow (1878) listed *Ligula reptans* Diesing from the subcutaneous musculature of the host, and *P. simoni* Rausch, 1949 has been described from the small intestine.

Comparison of *P. rugovaginosus* n. sp. with apparently closely related species is involved since there is disagreement as to the relative importance of certain morphological features used to separate species of *Paradilepis* Hsü, 1935 from those of the hymenolepid genus *Oligorchis* Fuhrmann, 1906. In addition some workers have either ignored or been unaware of the erection of the genus *Paradilepis* to include certain forms which previously would have been put in *Dilepis*. In view of this, a discussion covering these points is included here, before comparing the new species with others included in the genus.

The genus *Paradilepis* was established by Hsü (1935) to include those dilepids with: 1) a double crown of hooks, 2) little marked external segmentation, 3) sexual ducts dorsal to the excretory vessels and lateral nerve, and 4) testes few in number located on both sides of the female glands. Joyeux and Baer (1950) broadened this concept of the genus since they included in it forms which had pronounced external segmentation and testes largely posterior to the female glands. On the other hand they restricted the genus to forms with three to five testes. Fuhrmann (1932) in his most recent definition of *Oligorchis* included in it those HYMENOLEPIDIDAE which have: 1) a single crown of hooks, 2) four to eleven testes (he erroneously lists it as seven, but this would exclude another species he described in 1913 which has eleven testes), 3) internal and external seminal vesicles, and 4) genital ducts which pass dorsal to the excretory ducts.

Mayhew (1925) and Johri (1934, 1941), who have been followed by Southwell and Lake (1939), Neiland (1952) and in part by Wardle and McLeod (1952).

placed great stress on the restricted number of testes and included in *Oligorchis* all species with four testes, including forms which have a double crown of hooks and are without an external seminal vesicle. This would require an emendation of the description of the family HYMENOLEPIDIDAE since the presence of an external seminal vesicle and a single crown of hooks are usually considered fundamental family characters (Fuhrmann, 1932). It is felt here that, at least for the present, the definition of *Oligorchis* as laid down by Fuhrmann (1932) should be retained. It may be that certain exceptional hymenolepids may be found which have a double crown of hooks and yet otherwise appear to belong in the HYMENOLEPIDIDAE (see Joyeux and Baer, 1950). Thus the structure of the vas deferens is a key to the separation of those species which seem to share certain characters common to both *Oligorchis* and *Paradilepis*. This assumes the convergent evolution of these two genera by a reduction of the number of testes in each case and the retention without major modification of the basic structure of the vas deferens or the external seminal vesicle. Such an evolution seems likely since species of *Dilepis* and closely related genera, including *Paradilepis,* have a long and generally highly convoluted vas deferens, whereas species of *Hymenolepis* and closely related genera, including *Oligorchis,* have a short vas deferens modified into a pronounced external seminal vesicle.

The six species of *Paradilepis,* and synonyms with one exception, listed as valid by Joyeux and Baer (1950) are accepted here. It is proposed to reinstate one synonym as a valid species and to transfer to *Paradilepis* three other species previously assigned to either *Dilepis* or *Oligorchis.* These four species and their synonyms are:

Paradilepis burmanensis (Johri, 1941) n. comb.
Syn. *Oligorchis burmanensis* Johri, 1941
Host *Phalacrocorax javanicus*
Paradilepis longivaginosus (Mayhew, 1925) n. comb.
Syn. *Oligorchis longivaginosus* Mayhew, 1925
Host *Pelecanus erythrorhynchos*
Paradilepis minima (Goss, 1941) n. comb.[1]
Syn. *Dilepis minima* Goss, 1941
Hosts *Phalacrocorax varius, Microcarbo melanoleucus, Phalacrocorax ater*
Paradilepis yorkei (Kotlán, 1923) n. comb.
Syn. *Dilepis yorkei* Kotlán, 1923; *Oligorchis yorkei* (Kotlán, 1923) Mayhew, 1925
Host *Megapodius brunneiventris*

Oligorchis burmanensis Johri, 1941 was included by Joyeux and Baer (1950) as a synonym of *P. kempi* (Southwell, 1921), but without explanation. The two species have hooks of essentially the same size and number, although they are of slightly different shape. There are, however, several outstanding differences. *P. kempi* was described as having three testes, small and insignificant cirrus pouch, and a conspicuous vagina dorsal to the vas deferens, whereas *O. burmanensis* was

[1] Goss also described a second species, *Dilepis maxima* Goss, 1941. This worm has four testes and in other respects seems allied to *Paradilepis,* except that the cirrus is armed at the base with two spines 0.03 mm. long as in the genus *Gryporhynchus* Nordmann, 1832. However, the genital ducts pass dorsal to the excretory canals rather than between them as in *Gryporhynchus.* This worm requires re-examination because of its intermediate position.

described with four testes, cirrus pouch extending a little over half the breadth of the proglottid, and a vagina ventral to the cirrus sac. Even if one accepts that the testis number is variable, as Joyeux and Baer (1950) point out in the case of *P. urceus,* it is proposed that the differences of the vagina and cirrus are sufficient to reinstate *P. burmanensis* as a valid species.

Joyeux and Baer (1935) suggested that *Oligorchis longivaginosus* Mayhew, 1925 be transferred to *Paradilepis,* but they did not make this transfer. Through the kindness of the Bureau of Animal Industry the writer has been permitted to examine six slides of Mayhew's material, lot numbered 51182. The slides were originally designated by Mayhew as 25.22 (1) (toto mount) and 25.23 (1), (2), (3), (4), and (5) (five slides of serial sections). Since the rostellum (toto mount) bears no hooks their number and arrangement was not verified. Mayhew concluded that there was a single crown, although he admitted that there appeared to be two rows on the retracted scolex he examined. He counted a maximum of 20 hooks, but was uncertain of the total number. Thus the number and arrangement of hooks remains open to question. The hook shape shown by Mayhew is similar to that of hooks from most other worms presently included in the genus *Paradilepis.* The material, as Mayhew pointed out, was not well fixed and was difficult to interpret. Little of the internal structure could be seen in the toto mount, but some idea, particularly a picture of the sexual ducts, was obtained by reconstruction of the serial sections. The vagina is more variable than was indicated by Mayhew, being shorter and less convoluted in some series of sections. The discovery of a long and highly convoluted vas deferens, especially on slide 25.23 (2), and the absence of any structure resembling an external seminal vesicle, together with what has already been stated above, suggests that this worm should be reassigned to *Paradilepis.*

Dilepis minima Goss, 1941 clearly belongs in the genus *Paradilepis* as modified by Joyeux and Baer (1950). The rostellar hooks are similar in size and shape to those of *P. urceus* (Wedl, 1855), but the presence of a large cirrus pouch, and a strongly armed cirrus 0.24 mm. long (when everted as long as or longer than breadth of proglottid) appears to differentiate it from *P. urceus.* Study of type material may alter this decision.

Dilepis yorkei Kotlán, 1923 was transferred to the genus *Oligorchis* by Mayhew (1925) because the worm was described as having four testes. This action was accepted by Johri (1934, 1941), Southwell and Lake (1939), Polk (1942), and Neiland (1952); Fuhrmann (1932) and Wardle and McLeod (1952) retained the worm in *Dilepis.* The worm was described as having 50 to 52 large hooks in two rows, and a wide vas deferens of one or two coils. The large number of hooks in two rows suggests that this worm belongs in the Dilepididae. The transfer of this species to *Paradilepis* is further indicated, since Kotlán, the only worker to report the specimens, did not describe an external seminal vesicle.

Paradilepis rugovaginosus n. sp. can be distinguished from all others in the genus by the peculiar rugate structure of the vagina with the long cilia arranged to correspond with the rugae. This, plus the distinct strobilar segmentation, the presence of only four testes and their arrangement in each segment, and the fact that genital primordia are visible much farther from the scolex (15 mm. instead of 2 mm.) readily separate it from *P. simoni,* the other species reported from the osprey. The hooks of *P. simoni* and *P. rugovaginosus* are of similar shape and

size, but there is a small difference in number, namely, about 36 in the former and 32 in the latter.

The following key, modified from Joyeux and Baer (1950), is suggested for separating the species of *Paradilepis:*

1. Length of strobila 10 mm. or less 2
   Length of strobila exceeding 10 mm. 4
2. External strobilar segmentation absent or difficult to determine, testes usually 4 ..... *scolecina* (Rudolphi, 1819)
   External strobilar segmentation clearly visible, testes usually 3 3
3. Rostellar hooks 20, cirrus pouch longer than half width of proglottid. *minima* (Goss, 1941)
   Rostellar hooks 10 to 18, cirrus pouch less than half width of proglottid. *urceus* (Wedl, 1855)
4. Large hooks more than 0.300 mm. in length. *macracantha* Joyeux and Baer, 1935
   Large hooks less than 0.300 mm. in length 5
5. Number of rostellar hooks 50 to 52 ..... *yorkei* (Kotlán, 1923)
   Number of rostellar hooks fewer than 50 6
6. Number of rostellar hooks 20 to 22 7
   Number of rostellar hooks 30 to 36 10
7. All hooks nearly one size, 0.088 to 0.092 mm. in length. *longivaginosus* (Mayhew, 1925)
   Hooks of two sizes, large hooks 0.100 mm. or more in length 8
8. Number of testes 5, small hooks 0.080 to 0.087 mm. in length. *delachauxi* (Fuhrmann, 1909)
   Number of testes 3 or 4, small hooks 0.109 to 0.135 mm. in length 9
9. Testes 3, cirrus sac insignificant, vagina dorsal to cirrus sac. *kempi* (Southwell, 1921)
   Testes 4, cirrus sac up to half breadth of segment, vagina ventral to cirrus sac. *burmanensis* (Johri, 1941)
10. Hooks 36, external segmentation absent, usually 5 testes per segment, vagina not rugate ..... *simoni* Rausch, 1949
    Hooks 32, external segmentation distinct, usually 4 testes per segment, vagina rugate ..... *rugovaginosus* n. sp.

The consequence of some of these transfers is to reduce the genus *Oligorchis* to the following six valid species: *O. magnireceptaculata* (Johri, 1934); *O. nonarmatus* Neiland, 1952; *O. strangulatus* Fuhrmann, 1906; *O. toxometra* Joyeux and Baer, 1928; *O. kwangensis* Southwell and Lake, 1939; and *O. paucitesticulatus* Fuhrmann, 1913. For a complete list of species which have been included previously in the genus *Oligorchis* see Neiland (1952).

SUMMARY

Paradilepis rugovaginosus n. sp. from the osprey, *Pandion haliaetus carolinensis* (Gmelin), is described. The characteristics which differentiate *Paradilepis* and *Oligorchis* are discussed. It is pointed out that the presence of a long, convoluted vas deferens in the former and an external seminal vesicle in the latter are valuable

diagnostic features if others are not sufficiently distinct to make a separation. *Paradilepis longivaginosus* (Mayhew, 1925) n. comb., *P. minima* (Goss, 1941) n. comb., *P. yorkei* (Kotlán, 1923) n. comb., and *P. burmanensis* (Johri, 1941) n. comb. are proposed. A key is given for the separation of the species of *Paradilepis*.

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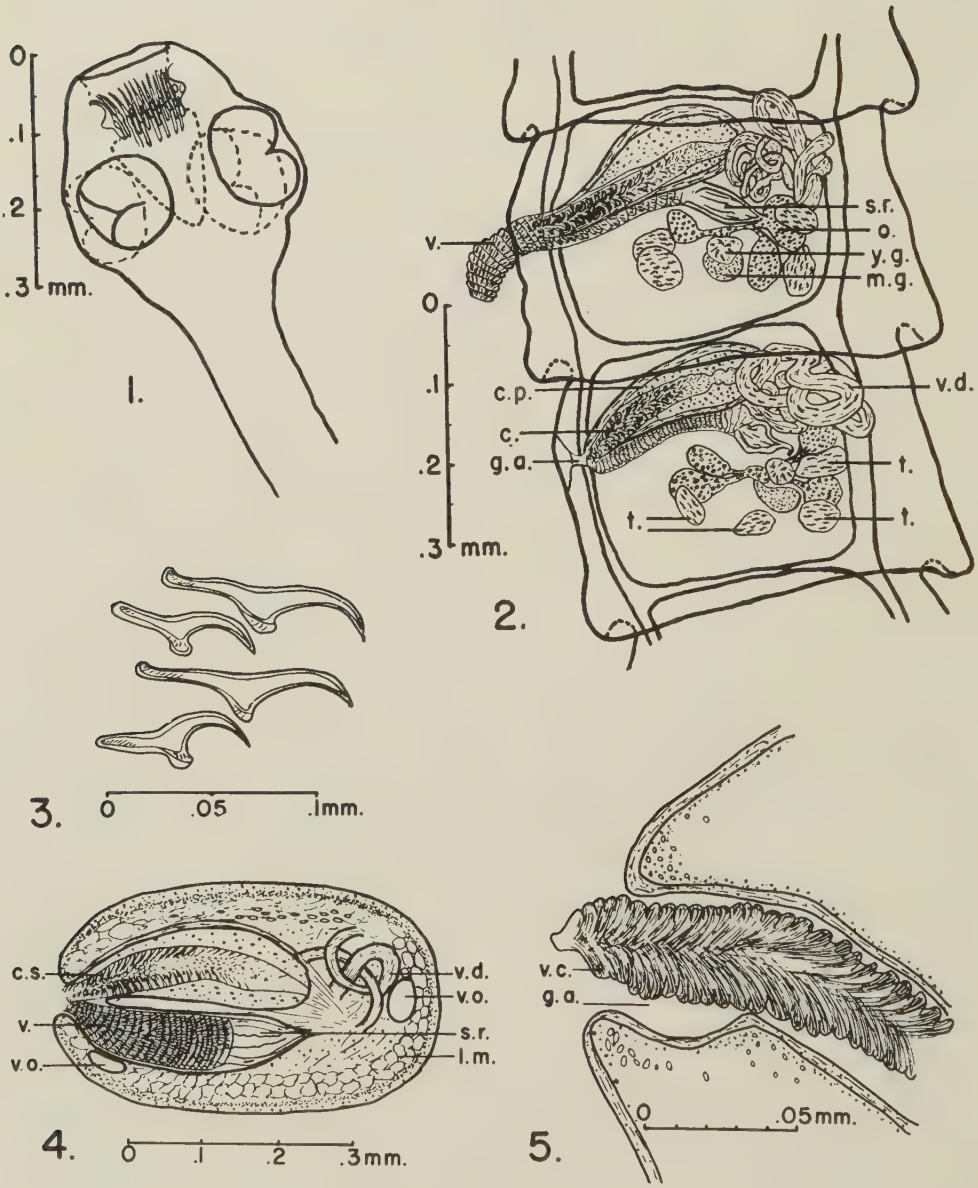
PLATE I



EXPLANATION OF PLATE

Paradilepis rugovaginosus n. sp., unless otherwise specified, figures were made with the aid of a *camera lucida*.

FIG. 1. Scolex.

FIG. 2. Dorsal view of two consecutive mature segments from whole mount: first segment with vagina everted, and second with vagina retracted.

FIG. 3. Large and small hooks.

FIG. 4. Cross-section, freehand and semidiagrammatic.

FIG. 5. Frontal section through distal part of vagina.

Abbreviations used in drawings: c. cirrus; c.p. cirrus pouch; c.s. cirrus spines; g.a. genital atrium; l.m. longitudinal muscle fibers; m.g. Mehlis' gland; o. ovary; s.r. seminal receptacle; t. testis; v. vagina; v.c. vaginal cilia; v.d. vas deferens; v.o. ventral osmoregulatory canal; y.g. yolk gland.

CERCARIA BRACHIDONTIS N. SP. FROM THE HOOKED MUSSEL IN LOUISIANA

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The hooked mussel *Brachidontes recurvus* (Rafinesque), also called *Mytilus recurvus* and *Mytilus hamatus,* is a very common associate of oysters (*Crassostrea virginica*) in brackish estuaries from Rhode Island to Texas. It usually grows on oysters (sometimes on other objects), to which it is attached by byssus threads, and reaches maximum size and abundance on the oyster beds of waters whose salinity is near the lower limit which oysters will tolerate. In such waters it becomes a serious competitor of the oyster, but apparently never replaces it as a dominant.

On July 26, 1951, hooked mussels were collected from experimental oyster trays in Bayou Wilkinson at the upper end of Barataria Bay, Louisiana. The next day, 40 of the larger mussels, 45 to 60 mm long, were dissected under a binocular dissecting microscope. Two specimens had the gonads completely destroyed and replaced by masses of orange-pigmented sporocysts which looked and moved like active fly maggots. Sporocysts also occupied part of the mantle tissue and had spread into other parts to some extent.

Most sporocysts were packed full of cercariae of the type called "Cercariaeum," no sign of a tail being present in mature larvae. Immature cercariae had a small knob on the posterior end which presumably represented the vestige of a tail. This cercaria is described below as:

Cercaria brachidontis n. sp.

Length of living or heat-killed specimens 0.33 to 0.43 mm., greatest width 0.14 to 0.18 mm. Oral sucker averaging about 0.075 mm in diameter, ventral sucker 0.09 mm and thus slightly larger than the oral sucker. Ventral sucker slightly posterior to the middle of the body length. Outer surface of body covered with very fine cuticular spines. No tail. No enlarged spines, stylet, or other structures specialized for penetration, on the anterior end. Six small cephalic glands on each side of pharynx, with ducts running dorsally over the oral sucker to the anterior end. Pre-pharynx very short, not usually visible in frontal view. Pharynx large and muscular, averaging 0.045 mm long and 0.035–0.040 mm wide. Esophagus approximately as long as pharynx. Intestinal crura reaching nearly to posterior tip of body, broad and conspicuous; epithelial cells of intestine filled with light-refractive granules or droplets. Reproductive organs represented by anlage of cirrus pouch, just anterior to ventral sucker, and anlagen of testes and ovary just posterior to ventral sucker. Excretory bladder consisting of a sack-like stem which ends posterior to gonad anlagen and ventral sucker, and a pair of narrow rami branching from each side of the anterior end of the stem, running forward to the level of the crural fork, and expanding into swollen bulb-like anterior ends. (There seem to be valves at the junction of these rami with the stem, so they may be collecting tubes rather than branches of the bladder.) Flame cell formula 2 $[(2+2)+(2+2)]$. Developing in sporocysts in gonad of *Brachidontes recurvus.* Type locality: Barataria Bay, Louisiana.

In dilute neutral red, cephalic glands stain very light brown or yellow, with unstained vesicular nuclei, and their ducts stain red. The granules or droplets in the intestinal wall stain yellow, and the contents of the lumen of the esophagus and intestine stain red. Genital anlagen stain pink.

This cercaria is similar to the tailless larvae which develop in marine bivalves and have been called the cercariae of *Gymnophallus* by European authors (see

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Dawes, 1946, for literature). It differs from them, however, in having long intestinal crura and a large ventral sucker. The tailless cercariae of the Zoogonidae, described by Palombi (1930) and Stunkard (1938, 1943), differ from all of these larvae in having a stylet and in several other morphological features, and develop in snails. The tailless cercariae in land snails (Brachylaemidae, etc.) and those in freshwater snails are excluded from comparison because of the differences in host and habitat. Some species of *Gymnophallus,* according to European authors, have a tailless cercaria borne in sporocysts, and the metacercariae also develop in marine bivalve molluscs. No metacercariae were found in any of the 40 *Brachidontes recurvus* dissected under a binocular microscope, nor have any been found in the hundreds of oysters which I have dissected. Therefore it seems improbable that *Cercaria brachidontis* uses bivalve molluscs as second intermediate hosts.

Cercaria milfordensis Uzmann, 1953, is very similar to *Cercaria brachidontis,* but a comparison of Uzmann's drawings with mine shows several differences.

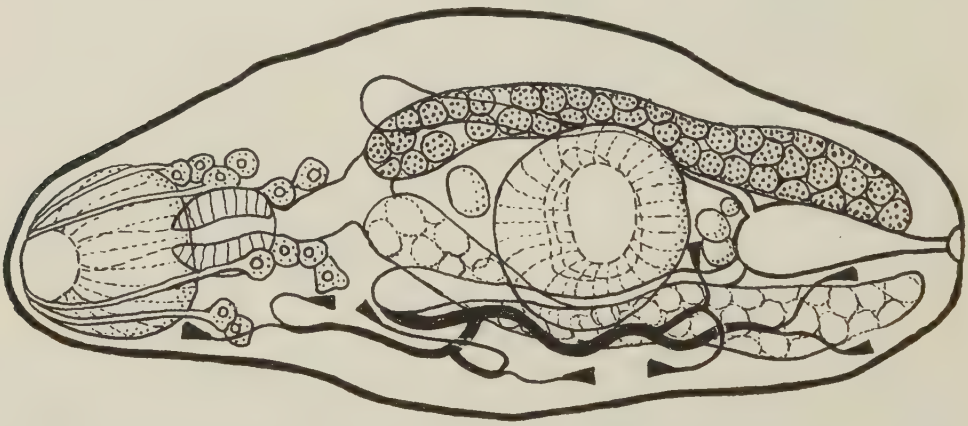

Fig. 1. *Cercaria brachidontis.* Dorsal view of living specimen treated with dilute solution of neutral red. Drawn with aid of a camera lucida; excretory tubules and flame cells drawn freehand.

Perhaps the most striking difference is the presence of a minute knob-like tail on *C. milfordensis.* An identical knob was seen on the posterior end of immature specimens of *C. brachidontis,* but not on the fully developed individuals. The gland cells of the two species also seem to be different. Uzmann tentatively referred *C. milfordensis* to the Fellodistomatidae, and it is not improbable that *C. brachidontis* also belongs to that family. *Tergestia* and *Gymnophallus,* representing two subfamilies of Fellodistomatidae according to Cable (1953), have the 2 [(2+2) + (2+2)] flame cell formula observed in *Cercaria brachidontis.*

This work was done at the marine laboratory of the Texas A. & M. Research Foundation at Grand Isle, Louisiana.

SUMMARY

Cercaria brachidontis, a new tailless cercaria with long intestinal caeca, with Y-shaped excretory bladder, with 2 [(2+2) + (2+2)] flame cell formula, and without a stylet or penetration spines, develops in active maggot-like orange-pigmented sporocysts in the gonads of the hooked mussel, *Brachidontes recurvus.*

The host mussel, a common associate and competitor of oysters in brackish estuaries, is completely castrated by the parasite. This cercaria may belong to the family FELLODISTOMATIDAE.

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A REVISION OF THE GENUS *PNEUMONYSSUS* (ACARINA: HALARACHNIDAE)

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The genus *Pneumonyssus* Banks, 1901, consists solely of mites parasitic in the respiratory tract of terrestrial mammals. The majority of known species are parasitic in old and new world monkeys, but species are known now from RODENTIA, CARNIVORA and HYRACOIDEA.

The biology of known members of the genus *Pneumonyssus* was considered by Oudemans (1935), together with an extensive review of the existing literature on the genus. The synonymy presented in that paper has been generally accepted in so far as the species known as of that date are concerned.

In general, *Pneumonyssus* species seem to be of low pathogenicity, *P. caninum* frequently occurring in the frontal sinuses of dogs with no accompanying symptomatology. In monkeys, nodular lesions of the lungs have been associated with the presence of mites of this genus, though frequently little or no gross pathology is noted. In infestations of *Macacca mulatta* with *P. simicola* at the Oakland, California, U. S. Naval Biological Laboratory, Major J. P. Ransom (1953, personal communication) reports that the general health of infested animals was good, although there were very evident gross lesions of the lungs caused by the mites.

Together with the genera *Halarachne* Allman, 1847, and *Orthohalarachne* Newell, 1947, *Pneumonyssus* constitutes the family HALARACHNIDAE Oudemans, 1906. The family diagnosis presented here represents a modification of that given by Baker and Wharton (1952).

HALARACHNIDAE

Diagnosis— Mites parasitic in the respiratory tracts of mammals. Body shape oval to wormlike. Dorsal plate of both male and female reduced and undivided,

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characteristically marked by insertions of muscles on inner surface; finely and densely punctate; without enlarged setae near posterior border. Tritosternum absent. Only 3 pairs of sternal setae present (possible exception of *Pneumonyssus stammeri* where Vitzthum (1930) illustrated a pair of metasternal setae). Sternal plate present. Jugularia absent. Genital plate of female reduced, rudimentary or absent. Genital opening of female a transverse slit between coxae IV. Genital opening of male at anterior margin of sternal shield. Anal opening posterior, on anal plate bearing 3 or 4 setae. Stigmata with small peritremes lateral to region of coxae IV. Tectum simple and triangular. Chelicerae with movable digit more strongly developed than fixed digit. Palps of 4 or 5 segments. Palpal tine present and bifurcate or reduced to a simple spinelet. All legs with protarsi, caruncles and claws.

The structure designated as pretarsus here, in accordance with the interpretation of Vitzthum (1929), is a terminal colorless portion of the tarsus bearing the caruncle and claws. It is usually distinguishable from the main body of the tarsus by a constriction. It should not be confused with the protarsus. The tarsus itself is subdivided, often incompletely, into a basal section correctly designated the protarsus by Chandler and Ruhe (1940), and a distal section, the tarsus proper.

In the modified family diagnosis of the HALARACHNIDAE given here, as in previous diagnoses (Baker and Wharton 1952, Newell 1947), there is little to distinguish it from the families RHINONYSSIDAE and ENTONYSSIDAE, parasites of the respiratory passages of birds and snakes respectively. In particular there is similarity with the ENTONYSSIDAE. A new species of *Pneumonyssus* described here seems to represent, in some respects, a link between HALARACHNIDAE and ENTONYSSIDAE; although by host relationship and major morphological features it definitely belongs in the former family, yet it has such typical entonyssid characters as a genital plate and an undivided dorsal plate of greater size than is typical for *Pneumonyssus*. Furthermore, the palpus is more similar to that found in certain *Entonyssus* and *Ophiopneumicola* species than to any of the described species of HALARACHNIDAE.

Since the new species of *Pneumonyssus* described here is represented by only a single specimen taken from a rodent, caution should be used in assessing its significance as a link between mammalian and snake-infesting forms. However, even if future investigation should show that the rodent is not the normal host of this species, the fact remains that here is a species intermediate between the HALARACHNIDAE and ENTONYSSIDAE.

Genus *Pneumonyssus* Banks

Pneumonyssus Banks 1901, Geneesk. Tijdschr. Nederl.-Indie, Vol. 41: 334–336. Type, by monotypy, *Pneumonyssus simicola* Banks.

Pneumotuber Landois and Hoepke, 1914, Centralbl. f. Bakt. Parasitenkde. u. Infekt., vol. 73, No. 6: 393. Type, by monotypy, *Pneumotuber macaci* Landois and Hoepke.

Diagnosis—Parasitic mites in the respiratory tracts of terrestrial mammals. Body oval, with not over three pairs of setae dorsally between posterior margin of dorsal plate and anal plate. Sternal plate present. Digitus mobilis of male chelicera varying from tubular and sinuate to hook-like and much enlarged over that of female; inflated lamella present or absent. Maxillary palpi four or five segmented, in some

appearing three segmented due to partial fusion of femur and trochanter; palpal tarsus fused with tibia or distinct as a small subterminal tibial eminence; bifid tarsal prong present or absent. Never with more than three pairs of ventral maxillary setae. Anterior peritremalia apparently absent.

KEY TO SPECIES OF *Pneumonyssus*

1. Palpi with 5 apparent segments 2
   Palpi with 3 or 4 apparent segments 4
2. Pretarsi of legs II, III and IV pedicellate; tarsal claws of leg I distinctly larger than those of leg III; last palpal segment an elongate tibio-tarsus 3
   Pretarsi of legs II, III and IV not pedicellate; tarsal claws of leg I subequal with those of leg III; palpal tarsus distinguishable as a small subapical lobe on the tibia. *Pneumonyssus bakeri*
3. First tarsal claws approximately ½ as long as tibia I; peritreme over 3 times as long as broad; metasternal setae absent ..... *Pneumonyssus caninum*
   First tarsal claws apparently as long as tibia I; peritreme not over 3 times as long as broad; metasternal setae present ..... *Pneumonyssus stammeri*
4. Claws of tarsus I of female much less developed than those of tarsus III 5
   Claws of tarsus I of both sexes developed about as much as those of tarsus III 6
5. Six pairs of dorsal plate setae; palpal prong distinct, bifurcate ..... *Pneumonyssus dinolti*
   Five pairs of dorsal plate setae; palpal prong reduced to a spinelet .. *Pneumonyssus duttoni*
6. Palpus with terminal pair of long setae; without long lateral basal seta on palpal tarsus .. 7
   Palpus with single long terminal seta; seta laterally at base of palpal tarsus at least twice the length of the segment ..... *Pneumonyssus procaviens*
7. Palpus less than ½ as long as the width of the basis capituli; claws of tarsus I measure approximately 20 μ in straight line from base to apex ..... *Pneumonyssus simicola*
   Palpus over ½ as long as the width of the basis capituli; claws of tarsus I measure approximately 37 μ in straight line from base to apex ..... *Pneumonyssus congoensis*

Pneumonyssus caninum Chandler and Ruhe

Pneumonyssus caninum Chandler and Ruhe, 1940, Jour. Parasitol. **26** (1): 60. [East Lansing, Michigan, ex *Canis familiaris* Linne; U. S. National Museum.]

The description of *P. caninum* by Chandler and Ruhe (1940) was based on adult female and larval forms collected in considerable number from the nasal region of a dog in Michigan. No male specimens were isolated at this time nor in the subsequent collections from dogs reported by Monlux (1940, 1951) and Monlux and Turk (1951).

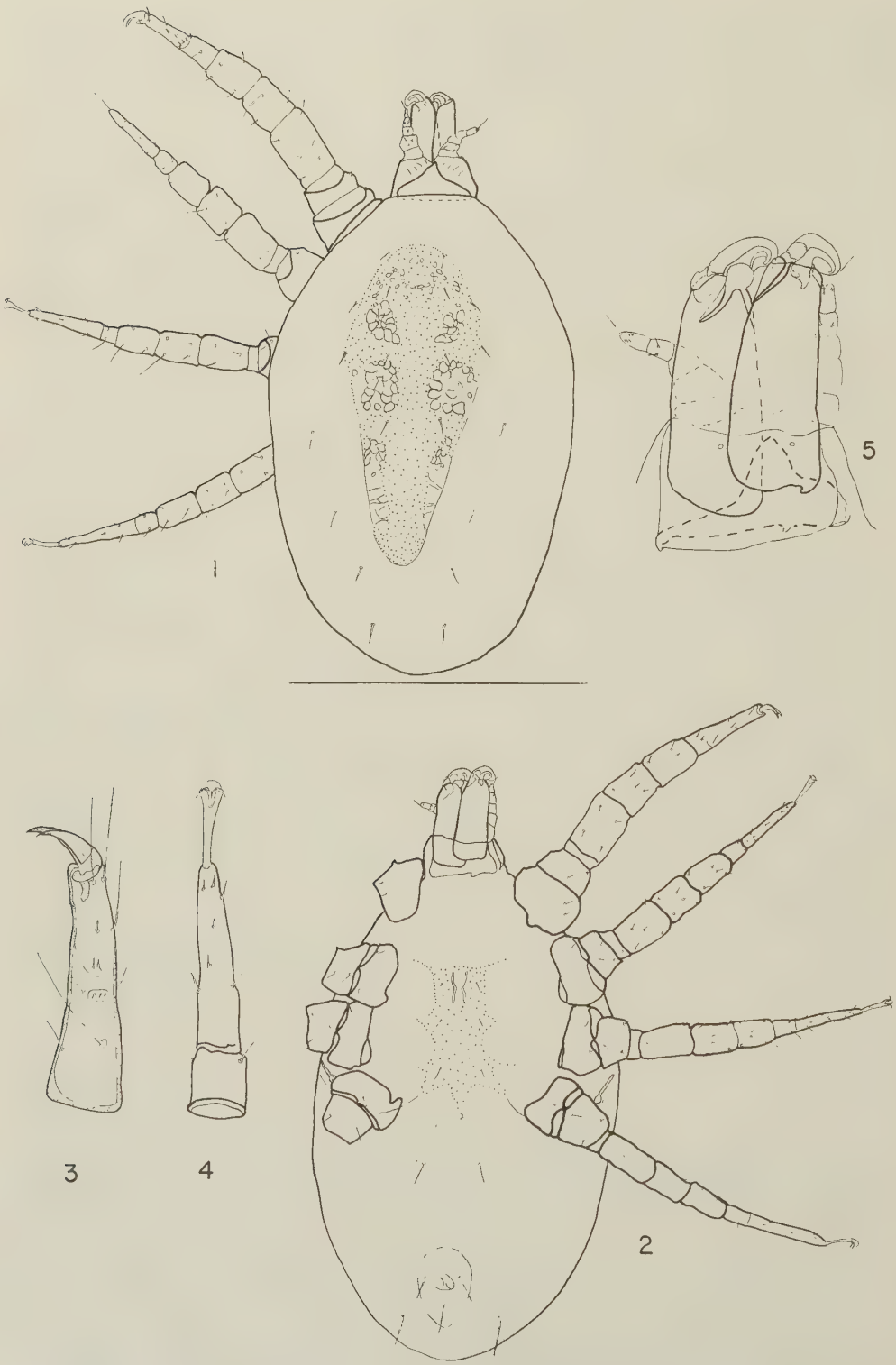
Recently a series of 14 specimens of *P. caninum* was obtained through the courtesy of J. R. Douglas and F. A. Ehrenford, who had collected them from the frontal sinuses of a dog at the Veterinary Science Clinic, University of California at Davis, California, on July 17, 1950. Douglas (1951) stated that no pathology was observed which could be attributed to the presence of these mites.

Included in this series are two males upon which the following description is based:

General body shape oval; idiosome length 990 microns, width 607 microns.

Gnathosoma roughly quadrangular, slightly narrower anteriorly; tectum a broadly triangular flap with a widely flaring base (Fig. 1). Palpi of five segments as illustrated (Fig. 5), with a single terminal spine; four to five spinelets, scarcely longer than wide at the base, present on tarsus; no tarsal tine. Chelicerae massive, heavily chitinized, much longer than palpi. Structure of tip somewhat obscure but basically consisting of an articulated, sclerotized, hook-like structure, the digitus mobilis, surrounded by a membranous bulbous sheath; digitus fixus, if present, vestigial.

Dorsum.—Dorsal plate roughly leaf shaped, finely porose (Fig. 1); length 616 microns, width 273 microns; margins indistinct in places; setal complex of dorsal plate consisting of six pairs of delicate setae, three pairs of which are marginal and may appear to be either on or off the plate. In the plesiotype specimen one seta of the anterior pair definitely off the plate



Pneumonyssus caninum, male. FIG. 1. Dorsum; FIG. 2. Venter; FIG. 3. Tarsus I, dorsolateral view; FIG. 4. Tarsus II, ventral view; FIG. 5. Gnathosoma, ventral view.

while the other is on. Four pairs of short dorsal setae, in addition to dorsal plate series, arranged in two posteriorly converging rows in the opisthosomal region; two pairs of setal complex posterior to dorsal plate.

Venter.—Male genital opening at the anterior apex of a flask-shaped structure medially situated betweeen coxae II, slightly caudad of the anterior border of the sternal plate. Sternal plate a very lightly sclerotized, finely porose structure, without clearly defined margins, as illustrated in Fig. 2; definitely on it, however, are three pairs of delicate setae located in two rows extending from the mid level of coxae II to the level of the posterior margins of coxae III. Anal plate roughly oval anteriorly extending to a blunt tip posteriorly, with three setae located as illustrated. Peritreme as described by Chandler and Ruhe (1940) for the female and as figured herein (Fig. 2). Only two pairs of setae on the opisthosome, only one pair of which located between level posterior margins of coxae IV and anterior margin of anal plate.

Legs.—Similar in general appearance to those of female. Anterior leg without visible pretarsus or caruncle, but with a pair of strong heavily sclerotized claws articulating subterminally with tarsus. Tarsus I with several long setae, short spines, and a mid-dorsal oval area containing three very short triangular spinules. This is probably homologous with Haller's organ of ixodids. Tarsi II to IV have long pretarsi, each ending in a caruncle and a pair of delicate recurved claws.

Location of type material.—Male plesiotype deposited in the U. S. National Museum together with a female from the same series. The remaining specimens are in the author's collection.

The female specimens of the California collection as well as a specimen taken from a dog at Ithaca, New York, and studied here, present some minor differences from the description of *P. caninum* given by Chandler and Ruhe (1940). A reduced and delicate sternal plate with irregular margins is present. This bears two pairs of setae with a third pair regularly located just off the posteromedial projection of the plate. On the ventral opisthosoma anterior to the anus there are only two pairs of setae on the unarmed portion as opposed to three pairs in the original description. On the dorsal plate the setal complex of six pairs is almost identical to that of the male except that the third pair of marginal setae is regularly off the dorsal plate proper. Only four pairs were found on this plate by Chandler and Ruhe. It is also apparent that the "microscopic, back-curving spines" of these authors are in reality minute pores of the dorsal plate. As in the male, tarsus I has a mid-dorsal oval area containing three very short triangular spinules.

Pneumonyssus bakeri n. sp.

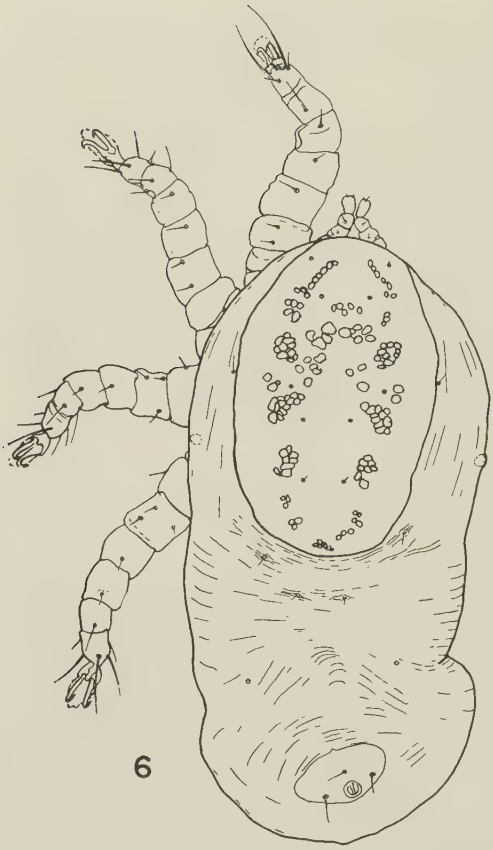
Female, Figs. 6–9

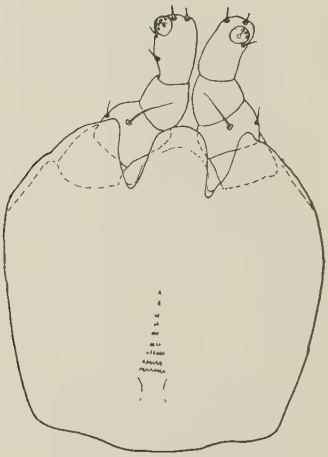
General shape elongate oval. Idiosoma 563 microns long and 273 microns wide.

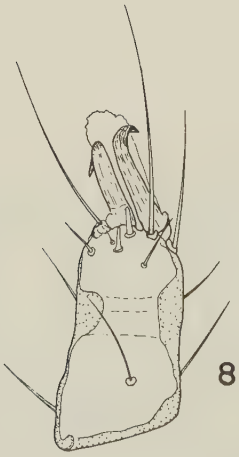
Gnathosoma roughly quadrangular, slightly narrowed at base. Vestigial capitular groove teeth arranged as illustrated on median-basal region of gnathosoma, ranging from eight in posterior to one in anterior rows. Antero-ventrally basis capituli terminates in three semitransparent membranous lobes. Palpi with five obvious segments, the tarsus represented by a subterminal lobe; usual forked tine of tarsus absent. Basal segment of palpus and most of 2nd segment covered ventrally by broadly triangular, membranous, maxillary lobes separated by a median hypostomal lobe. Tectum short and broadly triangular. Cheliceral structure indistinct.

Dorsum.—Dorsal plate oval, about 2/3 as wide as the body and extending posterior to coxae IV, measuring 295 microns by 215 microns wide; lightly sclerotized, with an irregular pattern of cell-like structures marking muscle insertions; with five pairs of minute pores with microsetae visible in some as illustrated. Three to four pairs of minute setae on unarmed cuticula as illustrated; fourth pair represented only by pores resembling setal sockets; cuticula finely striated. Anal plate probably terminally situated normally, but on type it is dorsal, broader than long and with usual three setae.

Venter.—Sternal plate irregularly quadrangular, broader than long, with three pairs of minute setae; margins of plate indistinct; plate characterized by delicate, scale-like design as illustrated. Vulva a transverse slit terminated by two small sclerites median to coxae IV. In type specimen upper lip of vulva is stretched and bowed forward, drawing the anterior portion







Pneumonyssus bakeri, female. FIG. 6. Dorsum; FIG. 7. Gnathosoma, ventral view; FIG. 8. Tarsus I, dorsal view; FIG. 9 Venter.

of the genital plate over the lower lip. Genital plate small, truncate and very lightly sclerotized; a pair of pores lateral to posterior margins. Two pairs of minute setae on opisthosoma as illustrated. Stigmata lying above and between coxae III and IV. Peritreme extremely short, almost as broad as long.

Legs.—Short, stout, with segmentation typical of the genus; caruncles and paired tarsal claws of all legs subequal; claws of tarsus I with subterminal tarsal articulation while each of those of other tarsi arises from base of colorless pretarsus distal to tarsal tip. Dorsum of tarsus I with subterminal complex of two blunt and two pointed spinules, and one pointed microspine in addition to three long terminal setae (Fig. 8); a single basal seta completes the dorsal tarsal setation.

Type.—Described from single female collected from *Tamiasciurus douglasii mollipilosus* (Audubon and Bachman) in Humboldt County, California, on June 13, 1951, by D. J. Gould and D. P. Furman. Deposited in the U. S. National Museum.

The specimen was collected by washing the rodent in a detergent solution. Doubtless the habitat was the respiratory tract of the host in common with other *Pneumonyssus* species.

The species is named for Dr. E. W. Baker, able and highly esteemed acarologist.

P. bakeri fits the family diagnosis of HALARACHNIDAE as given by Baker and Wharton (1952) except that the hypostome is not pilose, the palpal tine is absent, and tarsus I appears to have at best a vestigial pretarsus; however, these characteristics are shared with certain other *Pneumonyssus* species such as *P. caninum* and *P. duttoni,* and the family diagnosis given here has been modified accordingly. It may be separated from all other species of *Pneumonyssus* by its sternal plate, which is broader than long, by the presence of a definite though moderately reduced genital plate, and by the existence of a ventral subterminal lobe representing the tarsus, arising on the penultimate palpal segment.

P. bakeri may well represent a genus distinct from *Pneumonyssus* in view of the above points of difference from other members of the genus. The segmentation of the palpus is very similar to that found in the entonyssid, *Ophiopneumicola colubri* Hubbard and also in *Entonyssus glasmacheri* Vitzthum and the male of *Entonyssus vitzthumi* Schmidt; however, it differs from them in size and setation of the dorsal plate as well as in other characters.

A distinct genus is not established for *P. bakeri* at this time since, as indicated earlier, there exists the possibility inherent in any description based on a single known specimen that the true relationship is not known; the specimen possibly may only represent a "stray" on the recorded host. However, in view of the very close relationship of the respiratory mites of the families RHINONYSSIDAE, ENTONYSSIDAE and HALARACHNIDAE from birds, snakes and mammals, respectively, it is not surprising to find a species from a rodent which resembles in several respects certain of the snake-infesting species.

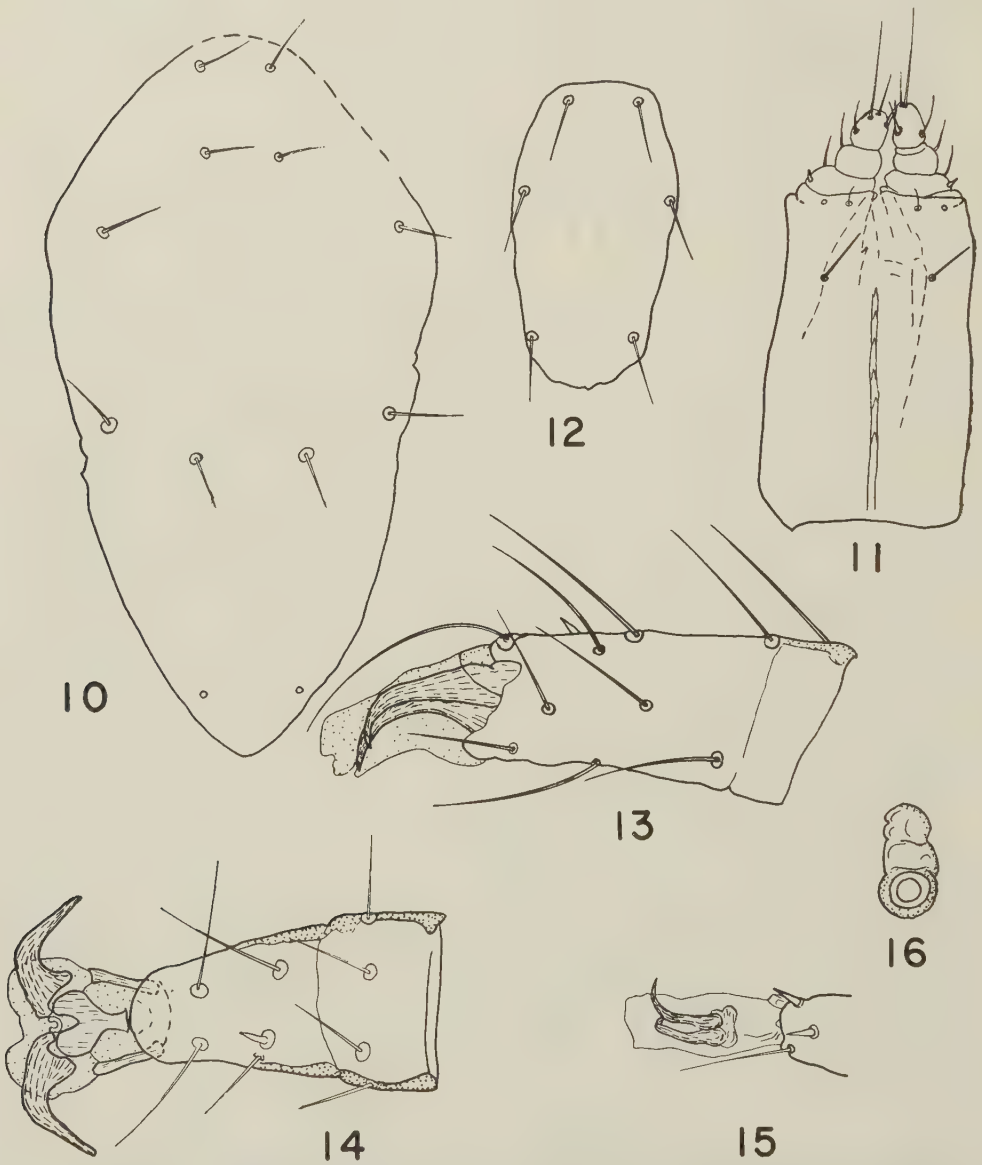
Pneumonyssus congoensis Ewing

Pneumonyssus congoensis Ewing, 1929, Proc. Ent. Soc. Wash. **31** (7): 129 [Belgian Congo, Africa, on *Papio* sp.; U. S. Nat. Mus.].

Since the original description of *P. congoensis* by Ewing in 1929 there have been significant changes in concepts as to characteristics specifically distinctive. For that reason, a redescription accompanied by drawings is given here based on study of a gravid cotype female:

Egg-shaped body widest just posterior to coxae IV. Idiosoma 924 microns long by 572 microns wide.

Gnathosoma—(Fig. 11) Quadrangular, 84 microns long by 50 microns wide, exclusive of palpi; a single median longitudinal row of teeth in the capitular groove (hypostome of some authors); three ventral pairs of maxillary setae (outer anterior pair represented only by pores in paratype). Palpus, length 29 microns, over 1/2 width of basis capituli; with three apparent segments; basal segment subdivided, with two distinct lateral sclerites separated by an indenta-



Pneumonyssus congoensis, female. (Figs. 10 and 12 at lower magnification than remaining figures.) Fig. 10. Dorsal plate; Fig. 11. Gnathosoma, ventral view; Fig. 12. Sternal plate; Fig. 13. Tarsus I, lateral view; Fig. 14. Tarsus II, dorsal view; Fig. 15. Tarsus IV, dorsal view; Fig. 16. Peritreme.

tion; with a lateral conical spinule and a seta; second apparent segment about half as broad as first and slightly longer; bearing an anterolateral seta; third segment (tibio-tarsus) longer than broad, bearing a spine midway on the inner margin which appears bifurcate but the tips are indistinct; one outer proximal and two apical setae, the outer of which is approximately as long as the palpus. Chelicerae not distinct in cotype examined; original description states

". . . with two chelae, each being modified into a sharp, tapering piercer; outer chela with a prominent elbow near its base and a long, curving, lancelike distal part; inner chela smaller than outer and without the elbow." Tectum in form of an anteriorly directed isosceles triangle.

Dorsum.—Dorsal plate elongate egg shaped, 374 microns long by 198 microns wide, with five pairs of short, subequal setae (Fig. 10). Very few minute setae on unarmed cuticula, exact number on paratype in doubt.

Venter.—Sternal plate 155 microns long by 94 microns wide, rounded posteriorly, with three pairs of setae as illustrated (Fig. 12). Vulva a transverse slit between coxae IV. No genital plate. Anal plate at posterior tip of opisthosome, egg shaped; anal opening slightly more than 1/3 as wide as anal plate; paired anal setae slightly longer than the single posterior seta and situated slightly in front of anal opening. Stigmata between and dorso-lateral to coxae III and IV with peritreme 28 microns long by 15 microns wide (Fig. 16). One pair of minute setae on unarmed cuticula of opisthosome.

Legs.—Rather short but well developed with segmentation typical of genus. All tarsi with well developed paired claws and small caruncles; first pair of tarsal claws appressed, 37 microns long, arising subterminally on tarsus (Fig. 13); second and third pairs large, angulate, strongly divergent, arising in membranous pretarsus distal to tarsal tip (Fig. 14); claws of leg III measuring 27 microns in straight line from base to tip; fourth pair arising as second and third but much smaller and diverging only slightly (Fig. 15). No unusual setation of legs.

Differentiation of *P. congoensis* from *P. simicola* Banks: These species are very closely related. The characters given by Ewing and Banks are inadequate to differentiate them. They may be distingushed readily, however, in numerous respects. The palpi of *P. congoensis* are 29 microns long as compared to a range of 16 to 20 microns in Banks' species. The distal segment of the tarsus of *P. congoensis* has a basal lateral seta which is absent on *P. simicola.* The subdivision of the basal palpal segment is much more distinct in the former species. The basis capituli of *P. congoensis* is over one and one-half times as long (84 microns) as wide (50 microns), whereas in *P. simicola* the length (av. 62 microns) is subequal to the width (av. 56 microns). Claws of legs I and III of *P. congoensis* measured in a straight line from base to apex are 37 and 27 microns long respectively as compared with approximately 18 and 16 microns respectively for *P. simicola.* Among the characteristics held in common are the size and shape of the dorsal plate, length of sternal plate and general appearance of tarsal claws. The claws of tarsi II and III of *P. simicola* flare as in *P. congoensis* but orientation of the legs obscures the character in some specimens studied.

Pneumonyssus simicola Banks

Pneumonyssus simicola Banks, 1901, Geneesk. Tijdschr. Nederl. Indie, **41**: 334 [Batavia, Java, *ex Macaca nemestrina* (L)]; Vitzthum, 1931, Z. Parasitenkde. **4**: 48–74 [Morphology, biology systematics]; Oudemans, 1935, Z. Parasitenkde. **7**: 494 [Redescription larva, female].

Pneumonyssus griffithi Newstead, 1906, Mem. Liverpool Sch. Trop. Med. **18**: 48 [*ex Macaca mulatta* (Shaw)]; Vitzthum, 1931, Z. Parasitenkde. **4**: 61 [Syn. *P. simicola*].

Pneumotuber macaci Landois and Hoepke, 1914, Centralbl. f. Bakt., **73** (1): 393 [*ex Macaca mulatta* (Shaw), nymph and adult briefly described]; Oudemans, 1915, Ent. Ber. Bd. 4, n. **83**: 184 [diagnosis nymph]; Oudemans, 1915, Tijdschr. Vergel. Geneesk. **1**: 183 [Description nymph, syn. with *P. simicola*].

Pneumonyssus foxi Weidman, 1915, Jour. Parasitol. **2** (1): 44 [*ex Macaca mulatta* (Shaw)]; Vitzthum, 1931, Z. Parasitenkde. **4**: 61 (Syn. *P. simicola*).

The writer has examined specimens agreeing with the description of *P. simicola* from the following collections: numerous specimens *ex* monkey lung from U. S.

Army Chemical Center, Medical Division, Edgwood Arsenal, Md., collected January 1950 by D. W. Jenkins; two specimens from the U. S. Nat. Mus. labeled merely Java; seven specimens labeled *P. griffithi ex Macaca mulatta* at Saranac Lake, N. Y., June 1926; seven specimens *ex* "Rhesus Monkey" at San Juan, P. R. collected in 1939 and 1942; five specimens *ex Macaca mulatta* Bahia, Brazil, 1928; several specimens collected in 1953 by J. P. Ransom, from *Macaca mulatta* at the Oakland, California, Naval Biological Laboratory.

Pneumonyssus procaviens Radford

Pneumonyssus procaviens Radford, 1938, Ann. Transv. Mus., **19** (2): 147–52 [South Africa, *ex Procavia capensis albaniensis* Roberts: Albany Museum, Grahamstown, S. Africa].

Through the courtesy of Dr. C. D. Radford type specimens of the male, female and larva of *P. procaviens* were made available for study. A redescription of the female and male is presented here since recent findings stress the significance of characters not treated in the original description.

Female

Body broadly egg shaped, 704 microns long by 528 microns wide.

Gnathosoma.—Roughly quadrangular; basis capituli broader than long, somewhat narrowed anteriorly, terminating ventrally in a slightly convex arc, no capitular setae visible on type female studied, though a pair were seen by Radford. Palpi short, approximately 22 microns long, with four segments; distal segment a fused tibio-tarsus with a long, baso-lateral seta at least twice the length of segment, a stout mid-ventral spine reaching beyond tip of segment and located in normal position of forked tine of palpus, and a single long apical seta approximately as long as distal segment; penultimate segment bears a long lateral seta; 2nd palpal segment with two lateral setae, the inner long, the outer minute. Chelicerae with no visible fixed digit; movable digit in form of a broad based, short, conical claw only slightly curved at tip, seeming to form a piercing organ.

Dorsum.—Dorsal plate with five pairs of setae; plate shape irregularly elongate oval, extending posteriorly slightly behind level of coxae IV, approximately 1/3 width of body at level of greatest dorsal plate width. Anal plate broadly oval, almost subcircular, longer than wide, with 3 setae; dorsally situated in type examined but probably terminal normally. Three pairs setae on unarmed cuticula as illustrated by Radford (1938).

Venter.—Sternal plate shape as illustrated by Radford (1938), much longer than broad and bearing three pairs of subequal setae, which are longer than in Radford's figures; the first pair extend to base of 2nd pair. Plate finely punctate. Vulva a transverse slit between coxae IV. No genital or ventral plates. One pair small setae mid-ventrally on opisthosoma. Stigmata above and between coxae III and IV. Peritreme twice as long as broad (24 by 12 microns).

Legs.—Short, stout, with segmentation typical of genus. Pretarsus of leg I rudimentary, claws and caruncle arising subterminally on tarsus; claws of tarsi II–IV arising from colorless pretarsus distal to tarsal tip. Claws of legs I–III stouter and longer than those of leg IV; former measure 20 microns measured in straight line from base of tip as compared to twelve microns for the latter; claws of leg IV appressed as compared to flaring condition of II and III.

Male

Body 792 microns long by 374 microns wide.

Gnathosoma.—Shape of basis capituli as in female except that anterior ventral border is somewhat concave; two pairs minute setae along anterior ventral border. Chelicerae very large, extending past tip of palpi a distance over twice the palpal length, much as figured by Radford but following details are added: a row of fine tooth-like spicules around base of large movable digit; lamellate movable digit in addition to pointed thumblike prong bears two pointed distal lamellae broadly joined basally and curved to form a hood-like expansion over basal "thumb." Fixed digit vestigial, represented by a very small sclerotized, pointed rod rising medial to base of movable digit.

Dorsum.—As in female.

Venter.—Sternal plate as in female but has male opening located near anterior border; setae as described for female.

Legs.—As in female.

P. procaviens has many specific characters in common with *P. simicola* and *P. congoensis.* It is immediately separable by several features, however, such as the presence of a single long terminal palpal seta and very long lateral palpal setae; the distinctive nature of the male chelae is described above in detail.

Pneumonyssus duttoni Newstead and Todd

Pneumonyssus duttoni Newstead and Todd, 1906, Mem. Liverpool Sch. Trop. Med., XVIII: 41–44 [Kasongo District, Congo, Africa, *ex Cercopithecus nictitans schmidti* Matschie]; Oudemans, 1935, Z. Parasitenkde. **7** (4): 500–3 [Description larva and male]; Cooreman, 1946, Rev. Zool. Bot. Afr., **39** (4): 331–5 [Belgian Congo, *ex Cercopithecus ascanius ascanius* Audebert (may actually be *C. n. schmidti* Matschie), redescription and figure of female].

P. duttoni is similar to *P. simicola* and *P. congoensis* in many respects, but it may be distinguished by the relatively small tarsal claws of leg I compared to those of leg III of the female. In addition, there are four obvious segments of the palpus on *P. duttoni* as compared to only three on the other two species, in which the basal article is obviously composed of two incompletely fused segments.

Pneumonyssus dinolti Oudemans

Pneumonyssus dinolti Oudemans, 1935, Z. Parasitenkde., **7** (4): 503–8 [*ex Macaca mulatta* (Shaw), female only described].

P. dinolti, in common with others such as *P. duttoni* has undergone reduction of the palpus so that only four segments remain. Unlike those with a reduction of palpal segments it has a rather well developed palpus bearing a marked two pronged tarsal tine; the palpus is definitely longer than the width of the basis capituli.

Pneumonyssus stammeri Vitzthum

Pneumonyssus stammeri Vitzthum, 1930, Z. Parasitenkde., **2** (5): 606–611. [*Ex* South American monkey, *Lagothrix poeppigii* Schinz, given as *L. infumatus* (Wollaffe) by Vitzthum].

P. stammeri is closely related to *P. caninum,* another New World species. Although specimens of Vitzthum's species were not available for study, the original description indicates that the claws of tarsus I are as long as tibia I. In *P. caninum* the first tarsal claws are approximately one-half as long as the tibia. In addition, metasternal setae are illustrated by Vitzthum; these are absent in *P. caninum.*

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SUMMARY

The family Halarachnidae and the genus *Pneumonyssus* are redefined. A key to the eight known species of *Pneumonyssus* is given with descriptions of *P.*

bakeri, n. sp. and of the previously undescribed male of *P. caninum.* The interrelationships of *Pneumonyssus* species are considered with redescriptions of species. Evidence is presented linking the mammal-infesting *Pneumonyssus* species with the snake-infesting ENTONYSSIDAE.

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NITROFURAZONE FOR THE PREVENTION OF EXPERIMENTALLY INDUCED *EIMERIA TENELLA* INFECTIONS IN CHICKENS

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Nitrofurazone (5-nitro-2-furaldehyde semicarbazone), a nitrofuran compound, has been tested by several workers for its effectiveness against coccidial infections in chickens.

Harwood and Stunz (1949, 1949a) reported that a mash containing 0.011 per cent nitrofurazone, administered continuously for 8 or 9 days, starting at the time of inoculation, greatly reduced mortality from experimentally-induced cecal coccidiosis in chicks 2 to 3 weeks of age. When treatment was started later than 56 hours after inoculation, this concentration of the drug was less effective. When the concentration of the drug in the mash was increased to 0.022 per cent and fed continuously for 2 weeks to uninfected chickens, these workers found that although the growth of the birds was slightly retarded, there was no permanent set-back. In a more recent paper (1953), they reported good protection for 3-week-old birds fed 0.0055 per cent nitrofurazone mash for 6 days, starting at the time of inoculation. The same authors (1950, 1951) and Harwood (1951) reported excellent results in the prevention of mortality and weight loss during natural outbreaks of cecal coccidiosis, when nitrofurazone was fed continuously at concentrations of 0.0067 per cent and 0.0055 per cent. When fed intermittently, however, it gave only partial control.

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Swales (1950) found that a 6-day treatment with a mash containing 0.0055 per cent nitrofurazone, starting at the time of exposure of chickens to heavily-contaminated litter, or a 4-day treatment with 0.011 per cent, starting 72 hours after exposure, was of little benefit in controlling cecal coccidiosis. A concentration of 0.011 per cent of the drug in the mash, administered for 5 days, starting 48 hours after exposure reduced mortality somewhat.

Horton-Smith and Long (1952) reported that nitrofurazone, added to the mash at a level of 0.022 per cent and fed continuously for 7 days, markedly reduced mortality from cecal coccidiosis. When fed at one-half this level for 10 consecutive days to chicks that were kept on contaminated litter, infection with *E. tenella* was prevented. In addition, these authors noted that nitrofurazone retarded the rate of development of *E. tenella* and that it affected the generation-2 schizonts by reducing their size and in some cases destroying them completely. They also found that there was a marked reduction in the numbers of oocysts discharged by birds receiving nitrofurazone medication as compared with untreated control birds. Moreover, they found no evidence that nitrofurazone medication interfered in any way with the development of immunity.

Peterson and Hymas (1950) reported that the administration of 0.005 per cent to 0.03 per cent nitrofurazone in the mash, beginning at the time of infection, reduced death losses from *E. necatrix* and favorably modified the clinical nature of the disease. At a concentration of 0.04 or 0.05 per cent, they found the drug to be markedly toxic to the birds.

EXPERIMENTAL PROCEDURE

The birds used in these experiments were obtained as one-day-old chicks from the Poultry Section of the Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Center, Beltsville, Maryland.

Except for those birds placed on litter and given continuous treatment (experiments 6 and 7), all chicks were kept in electrically-heated brooders or wire-floored cages throughout the experiments. In experiments 6 and 7, the birds were infected by administering approximately equal numbers of sporulated oocysts of *Eimeria tenella* in the feed each day for 4 days. In all other experiments, sporulated *E. tenella* oocysts were introduced into the esophagus with a pipette.

Nitrofurazone was administered in the form of a commercial mix, which was added to the mash at levels of 0.1 and 0.2 per cent. By analysis, the amount of nitrofurazone present at these levels was 0.0108 per cent and 0.0216 per cent, respectively, except in experiment 7 in which the percentage of drug present was 0.0112 per cent. The period of medication was 5 to 10 days in experiment 3, 7 days in experiments 1, 2, 4, and 5, and 4 weeks in experiments 6 and 7. In all experiments, the nitrofurazone mash was kept before the birds continuously throughout the medication period.

EXPERIMENTAL RESULTS

Symbols are used to designate groups of birds and to indicate time of medication. The "T" indicates "treated" (i.e., medicated) group, and the number preceding or following it the day treatment was begun in relation to the time of inoculation. Thus "T0" indicates that treatment was started on the day of inoculation, "T1"

one day after inoculation, etc. Conversely, "1T" would indicate that treatment was started one day before inoculation, "2T" two days before inoculation, etc. These symbols are used in the tables as well as in the text.

The results indicate that the feeding of 0.0108 per cent nitrofurazone in the mash for 5 to 10 days was of little benefit, and that it was inconsistent in its effect upon *Eimeria tenella*. When fed continuously for a 4-week period at the above level, the drug proved to be only slightly efficacious. When fed for 7 days at a level

TABLE 1.—*Weight gains and mortality*

Expt. no.	Chicks		No. oocysts per chick	Treatment		Deaths from coccidiosis		Weight gain (grams)[2]
	No.	Age[1]		Medication	Time	No.	Per cent	
1	20	25	0	None		0	0	231
	20	25	0	0.0108	7 days	0	0	216
	20	25	150,000	None		7	35	192
	20	25	150,000	0.0108	2T, 7 days	2	10	193
	20	25	150,000	0.0108	T0, 7 days	0	0	201
	20	25	150,000	0.0108	T1, 7 days	1	5	202
	20	25	150,000	0.0108	T2, 7 days	2	10	200
	20	25	150,000	0.0108	T3, 7 days	7	35	187
	20	25	150,000	0.0108	T4, 7 days	6	30	182
	20	25	150,000	0.0108	T5, 7 days	5	25	179
2	20	22	0	None		0	0	251
	20	22	0	0.0108	7 days	0	0	241
	20	22	127,000	None		10	50	141
	20	22	127,000	0.0108	2T, 7 days	3	15	191
	20	22	127,000	0.0108	T0, 7 days	2	10	193
	20	22	127,000	0.0108	T1, 7 days	1	5	202
	19	22	127,000	0.0108	T2, 7 days	5	26	187
	20	22	127,000	0.0108	T3, 7 days	4	20	138
	20	22	127,000	0.0108	T4, 7 days	9	45	153
	20	22	127,000	0.0108	T5, 7 days	8	40	146
3	19	17	0	None		0	0	297
	20	17	130,000	None		10	50	255
	19	17	130,000	0.0108	2T, 7 days	9	47	235
	20	17	130,000	0.0108	2T, 9 days	7	35	239
	18	17	130,000	0.0108	2T, 10 days	11	61	239
	20	17	130,000	0.0108	T0, 5 days	7	35	272
	20	17	130,000	0.0108	T0, 7 days	6	30	267
	20	17	130,000	0.0108	T1, 5 days	7	35	246
	20	17	130,000	0.0108	T1, 7 days	5	25	255
4	19	13	0	None		0	0	159
	20	13	0	0.0216	7 days	0	0	145
	20	13	132,000	None		12	60	98
	20	13	132,000	0.0216	2T, 7 days	4	20	99
	20	13	132,000	0.0216	T0, 7 days	0	0	130
	20	13	132,000	0.0216	T1, 7 days	0	0	132
	20	13	132,000	0.0216	T2, 7 days	1	5	120
	20	13	132,000	0.0216	T3, 7 days	7	35	117
	20	13	132,000	0.0216	T4, 7 days	9	45	100
	20	13	132,000	0.0216	T5, 7 days	12	60	95
5	19	16	0	None		0	0	158
	20	16	147,000	None		8	40	99
	20	16	147,000	0.0216	2T, 7 days	1	5	114
	20	16	147,000	0.0216	T1, 7 days	2	10	127
	20	16	147,000	0.0216	T2, 7 days	1	5	114
	20	16	147,000	0.0216	T3, 7 days	2	10	109
6	90	20	oocysts in feed	0.0108	28 days	20	22	354
	90	20	oocysts in feed	None		32	36	367
7	98	21	oocysts in feed	0.0112	28 days	28	29	312
	99	21	oocysts in feed	None		48	48	329

[1] Age is age of chicks in days on day of inoculation.
[2] Weight gain is average per bird of surviving birds.

of 0.0216 per cent, mortality was reduced and weight losses due to *E. tenella* were checked, provided the drug was administered coincident with or within 48 hours after the ingestion of sporulated oocysts. Medication at the 0.0216 per cent level did, however, interfere with weight gains when fed to uninoculated birds for a 7-day period, thus showing that the drug was toxic at this concentration.

Mortality.—As shown in Table 1, the mortality among chickens medicated at 0.0108 per cent was erratic. In general, the T0 and T1 groups (medicated 7 days)

were benefited, but the low mortality in these groups in experiments 1 and 2 was not duplicated in experiment 3. The 2T group (medicated 7 days) in experiments 1 and 2 also showed relatively low mortality. However, in experiment 3, where treatment of this group was prolonged to 9 and 10 days, mortality was high, so that the continuation of medication to cover the sixth through the eighth days after inoculation proved to be of no benefit. The T3, T4, and T5 groups showed little benefit from nitrofurazone medication at this level.

When the medication was stepped up to 0.0216 per cent and administered for 7 days, mortality was markedly reduced in the T0, T1, and T2 groups. As compared to the inoculated controls, the 2T and T3 groups were also benefited in experiment 5, but not in experiment 4. The T4 and T5 groups, which were tested only once at this level of medication, were no better protected than they were at the lower level.

It should be noted that Horton-Smith and Long, when using nitrofurazone at levels of 0.02, 0.03, and 0.04 per cent for 7 days, also failed to achieve 100 per cent control of mortality.

Continuous medication for 4 weeks at levels of 0.0108 and 0.0112 per cent resulted in a reduction in mortality, but the death rate could not be considered low except in a relative sense.

Weight Gains.—Also shown in Table 1 are the weight gains of the surviving birds in each experiment. The standard deviation method for determining statistical significance was used in evaluating these data.

At a medication level of 0.0108 per cent, the results were inconstant. In experiments 1 and 3, no significant difference in growth among the various treated groups was noted when compared either with each other or with the inoculated controls. In experiment 2, however, the T0, T1, T2, and 2T groups made significantly better gains than the inoculated controls.

At the 0.0216 per cent medication level the best growth was made by the T0 and T1 groups, but in neither of these groups were the weight gains comparable to those of the uninoculated controls. However, the weight gains made by the T0, T1, and T2 groups in both experiments in which this medication level was used were significantly higher than those made by the uninoculated controls, while the weight gains made by the 2T, T4, and T5 groups were not. In the T3 group the difference was significant in experiment 4, but not in experiment 5. Benefit from medication at 0.0216 per cent was thus confined to those groups that were placed on treatment at the time of inoculation or not later than 48 hours after inoculation.

The uninoculated medicated controls failed to make as good gains as the uninoculated unmedicated controls. This difference was statistically significant at the 0.0216 per cent level of medication, but not at the 0.0108 per cent level.

Under a regimen of continuous medication at 0.0108 and 0.0112 per cent, the surviving controls made better growth than did the surviving medicated birds, but the difference proved to be without significance.

Hemorrhage.—The treated groups passed about the same amount of blood as the inoculated controls. There was a 24-hour delay in the appearance of severe hemorrhage in the T0, T1, and 2T groups, except in experiment 3, and less blood was passed by the T0 and T1 groups throughout the course of the infection in all experiments except experiment 3. It was also noted that in experiment 3 the mor-

tality was heavy, the weight gains poor, and the hemorrhage copious in all groups; in this experiment, none of the treated groups showed any great benefit from nitrofurazone medication. In every experiment, the treated birds continued to pass small amounts of blood for 1 to 5 days after the cessation of hemorrhage in the inoculated controls.

Oocyst Output.—Oocyst discharge in millions of oocysts per bird for the 2T, T3, and inoculated control groups is shown in Table 2. In all cases, the largest daily elimination of oocysts among the controls was on the seventh day after inoculation, namely, the first day of oocyst discharge, while on the eighth day there was a sharp drop in the number of oocysts passed. In experiments 4 and 5 the oocyst count on the ninth day was approximately equal to that on the eighth day, while in experiment 2 there was a decided reduction in oocyst elimination on the ninth day as compared to the eighth day. The over-all pattern was, however, similar enough so that the difference could be at least partly accounted for by a small difference in the timing of the collection of droppings. In the tenth day the oocyst count dropped to a low level, and continued to remain so each day thereafter.

The 2T groups were also fairly uniform in general pattern of oocyst discharge.

TABLE 2.—*Oocyst output in millions of oocysts per bird*

No. days after inoculation	Experiment 2 (0.0108)			Experiment 4 (0.0216)			Experiment 5 (0.0216)		
	Time of treatment			Time of treatment			Time of treatment		
	Controls	2T	T3	Controls	2T	T3	Controls	2T	T3
7	38.27	1.06	22.43	31.84	1.31	5.18	39.75	10.13	5.33
8	25.78	52.17	15.34	12.49	52.40	1.70	15.53	52.10	4.36
9	11.24	30.21	4.48	10.48	15.68	1.01	15.24	11.00	0.94
10	5.40	12.39	1.44	5.28	11.62	0.39	4.01	9.24	0.39
11	4.13	3.52	1.34	11.05	1.75	0.41	3.40	4.05	—
12	4.24	5.99	0.18						
13	5.60	2.98	0.54						

They passed a small number of oocysts on the seventh day, a very large number on the eighth day, and diminishing numbers thereafter.

In the T3 groups, as in the inoculated controls, the maximum oocyst shower occurred on the seventh day after inoculation. From the eighth day on, there was a steady decline in oocyst output.

There were striking differences in oocyst discharge, as regards both the time the birds were placed on medication and the strength of medication used. Birds placed on medication 2 days prior to inoculation in every case were 24 hours later in reaching their peak discharge of oocysts than either the controls or the birds placed on medication 3 days after inoculation. On the other hand, their heaviest daily oocyst discharge was much greater than the highest daily count obtained for the controls, and the total oocyst output for the 2T groups over the entire period for which counts were made was roughly equivalent to that of the controls, the essential point of difference thus being the 24-hour delay in the appearance of the largest oocyst shower and its greater volume when it did come. The T3 groups, on the other hand, while eliminating their maximum number of oocysts for a single day on the same day as the controls—the seventh day after inoculation—passed a much smaller number of oocysts than either the controls or the 2T groups. The 2T group which was treated with 0.0108 per cent nitrofurazone passed large numbers of oocysts on both the eighth and ninth days after inoculation, but the 2T

groups which were treated with 0.0216 per cent discharged large numbers of oocysts only on the eighth day. The oocyst output of the T3 birds on 0.0216 per cent nitrofurazone was markedly less than that of the T3 birds receiving 0.0108 per cent medication.

TABLE 3.—*The effect of nitrofurazone on the severity of cecal lesions*

Time of medication	No. birds with cecal lesions of grade*					Total no. birds	Ave. grade of lesions
	0	1	2	3	4		
Experiment 1. 0.0108 per cent							
2T, 7 days	0	7	10	1	2	20	1.90
T0, 7 days	1	8	11	0	0	20	1.50
T1, 7 days	4	7	7	1	1	20	1.40
T2, 7 days	1	5	11	1	2	20	1.90
T3, 7 days	0	2	9	2	7	20	2.70
T4, 7 days	2	3	8	1	6	20	2.30
T5, 7 days	1	2	7	5	5	20	2.55
Control	0	1	10	2	7	20	2.75
Experiment 2. 0.0108 per cent							
2T, 7 days	0	1	16	0	3	20	2.25
T0, 7 days	1	0	15	2	2	20	2.20
T1, 7 days	1	1	16	1	1	20	2.00
T2, 7 days	0	2	9	3	5	19	2.58
T3, 7 days	0	0	12	4	4	20	2.60
T4, 7 days	0	1	6	4	9	20	3.05
T5, 7 days	0	1	7	4	8	20	2.95
Control	0	0	5	5	10	20	3.25
Experiment 3. 0.0108 per cent							
2T, 7 days	5	4	1	0	9	19	2.21
2T, 9 days	2	6	5	0	7	20	2.20
2T, 10 days	1	4	2	0	11	18	2.89
T0, 5 days	4	8	1	0	7	20	1.90
T0, 7 days	3	9	2	0	6	20	1.85
T1, 5 days	4	7	2	0	7	20	1.95
T1, 7 days	2	9	4	0	5	20	1.85
Control	1	6	3	0	10	20	2.60
Experiment 4. 0.0216 per cent							
2T, 7 days	0	1	15	0	4	20	2.35
T0, 7 days	0	1	19	0	0	20	1.95
T1, 7 days	1	2	17	0	0	20	1.80
T2, 7 days	3	2	14	0	1	20	1.70
T3, 7 days	0	1	12	0	7	20	2.65
T4, 7 days	0	0	11	0	9	20	2.90
T5, 7 days	0	0	8	0	12	20	3.20
Control	1	0	7	0	12	20	3.10
Experiment 5. 0.0216 per cent							
2T, 7 days	0	0	18	1	1	20	2.15
T1, 7 days	1	1	15	1	2	20	2.10
T2, 7 days	1	1	16	1	1	20	2.00
T3, 7 days	1	1	13	3	2	20	2.20
Control	0	0	9	3	8	20	2.95
Experiment 6. 0.0108 per cent							
28 days continuous	30	9	17	0	0	56	0.76
Control	11	4	34	4	0	53	1.58
Immunity test control	0	0	8	6	6	20	2.90
Experiment 7. 0.0112 per cent							
28 days continuous	4	8	11	0	0	23	1.30
Control	3	3	16	0	0	22	1.59
Immunity test control	0	0	24	5	8	37	2.57

* Grade of lesions: 0—no lesions; 1—barely recognizable lesions; 2—cecal walls thickened; small to medium cores; 3—cecal walls very thick; large cores or much blood; 4—bird died of coccidiosis.

Post-mortem Findings.—At necropsy each bird was graded according to the severity of the cecal lesions present and placed in one of 5 categories, as shown in Table 3. The average grade of lesions for each group was calculated by multiplying

the number of birds falling within each grade by the corresponding grade number, totalling the products, and dividing the total thus obtained by the number of birds in the group. Although these figures were arrived at arbitrarily, they are of use in determining the effectiveness of nitrofurazone in reducing cecal lesions.

A comparison of the figures for the average grade of lesions shows that when medication was administered at a 0.0108 per cent level for a 7-day period the T0 and T1 groups were least severely affected by the disease, the 2T and T2 groups were affected somewhat more severely, while the T3, T4, and T5 groups were hit nearly as hard as the controls. At the 0.0216 per cent level, the T0, T1, and T2 groups were distinctly less affected than any other treated groups in experiment 4, but in experiment 5 there was little difference among any of the treated groups, all being much less affected than the controls.

Test for Immunity.—In the continuous feeding experiments, the birds were tested for acquired immunity at 49 days of age by inoculation with a challenge dose of approximately 250,000 oocysts per bird. In addition, a number of birds of the same hatch, which had neither been exposed to *Eimeria tenella* nor received medication of any kind (immunity test controls), were given the same challenge dose. In both experiments the medicated birds were less severely affected than the unmedicated ones, while both medicated and unmedicated groups were much less affected than were the immunity test controls. No mortality in either previously exposed group resulted from administration of the challenge inoculation, but there was a mortality of 30 and 22 per cent, respectively, among the immunity test controls.

CONCLUSIONS AND SUMMARY

Mortality, weight gains, hemorrhage, oocyst output, and post-mortem records indicate that the administration of nitrofurazone for 7 days at a level of 0.0216 per cent, starting at, or within 48 hours after, inoculation, exercised a beneficial effect on chicks experimentally infected with *Eimeria tenella* oocysts.

Nitrofurazone administered at a level of 0.0216 per cent for 7 days, starting 48 hours before infection or more than 48 hours after infection, gave only slight benefit. When treatment was delayed until the appearance of symptoms of coccidiosis, the beneficial effect of the drug was negligible.

Nitrofurazone administered at a level of 0.0108 per cent for 7 days was only slightly beneficial, and its effects were inconsistent.

Nitrofurazone administered at a level of 0.0108 or 0.0112 per cent continuously for 28 days gave partial protection against cecal coccidiosis. It did not interfere in any way with the development of resistance, but apparently enhanced it.

Nitrofurazone at a level of 0.0108 per cent had no significant effect on the growth rate of chicks, but at 0.0216 per cent an adverse effect was evident when fed for a 7-day period.

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THE EFFECT OF MAKLUA (*DIOSPYROS MOLLIS*) IN THE TREATMENT OF HUMAN HOOKWORM[1]

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Hookworm infection is very common in Thailand (Barnes, 1918, O'Brien, 1923, Sadun, 1953, Sadun *et al.,* 1953), and is often encountered concurrently with *Ascaris lumbricoides.* Since hexylresorcinol is regarded as the safest and most effective ascaricide (Faust, 1939) and is quite effective against hookworm, it is the drug of choice among the local medical authorities.

Although it has many advantages over known helminthicides, mass treatment with hexylresorcinol is not practicable in Thailand because it is very expensive by local standards and because many people do not return to the clinics after treatment to receive the administration of a purgative.

These considerations led to the search for a drug effective against hookworm which could be given in the presence of *Ascaris* infection, be readily available at a very low cost, and which does not need to be followed by a purgative.

Diospyros mollis, of the family EBENACEAE, is found almost everywhere in Thailand. The berries of this plant are popularly known as Maklua and are used extensively to impart a black coloration to silk (Anon., 1926). The fruiting season in Thailand is from May to February (Craib, 1926). The local herb doctors have used Maklua for the treatment of intestinal diseases as far back as it can be ascertained. During the last two decades, many general practitioners and members of the staff of the two University Hospitals in Bangkok have used this drug for the treatment of intestinal helminthic infections, but to our knowledge no results have ever been published. The only indirect reference to this drug appears in connection with the treatment of *Fasciolopsis buski* "by an unofficial but well known native antihelminthic drug, with good results" (Daengsvang and Mangalasmaya, 1941).

After numerous interviews with the members of the University Hospitals staff and with several local practitioners, the following information was uncovered.

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During 1933 and 1934, Dr. Phra Abhantrachat Pisal used Maklua routinely for the treatment of hookworm patients at Siriraj University Hospital, with good results. In 1936, Dr. Prayoon Saibamroong at Siriraj University attempted to study the therapeutic action of Maklua against *Ancylostoma caninum* in dogs. He reported excellent results as long as the berries were used when still completely green. If the berries were allowed to turn even partially black the therapeutic action was considerably lessened or lost. In 1938, Dr. Vek Netraviseth of the same University attempted to isolate the active principles. He obtained a powder of unknown chemical structure that when fed to experimentally infected dogs had a similar action to that of the freshly crushed berries. However, the powder was unstable and lost its therapeutic action after prolonged exposure to the air.

As far as toxicity of Maklua is concerned, no extensive studies have been carried out. The fact that this fruit has been widely used for a long time without reported untoward symptoms is often regarded as proof of its being harmless. Dr. Prayoon Saibamroong gave to dogs the equivalent of ten times the maximum therapeutic dose (as calculated on the basis of body weight) without detecting any symptom other than a mild drowsiness. Dr. Luang-li, at Chulalongkorn University, ingested the equivalent of 50 berries (twice the maximum dose used therapeutically) without noticing any ill effect. He also gave a dog ten times the usual therapeutic dose without observing any clinical or gross pathological signs. Dr. Vek Netraviseth gave to dogs large amounts of the extracted powder without observing outward toxic signs. Twenty-two doctors who have used Maklua for their patients have been interviewed. None reported having observed serious signs of toxicity. The only symptoms occasionally encountered were nausea, vomiting and mild drowsiness.

Investigation of the effects of Maklua in the treatment of human hookworm forms the basis of the present report, some results of which have been previously published in abstract form (Sadun and Vajrasthira, 1952).

MATERIALS AND METHODS

A total of 112 persons infected with hookworm were selected for this study. All of the patients were Thai living in Cholburi Province (Central Thailand). The feces were examined at least two times before treatment in order to establish a reasonable constancy of egg elimination. The results of the treatment were checked by examining the stools of all the patients after the administration of the drug. All stools were examined in two ways: One ml. was examined by the Willis-Malloy salt flotation concentration method (1921), and 4 ml. were mixed with a decinormal solution of sodium hydroxide for a quantitative determination of hookworm eggs according to the modified Stoll technique (Stoll and Hauscheer, 1926). In specimens which were Willis positive and Stoll negative, the egg count was arbitrarily figured as 100 eggs per ml. of feces (Cort *et al.*, 1929). All tests were carried out during the dry season when reinfection with hookworm was at a minimum.

Maklua was prepared and administered as follows: One thousand freshly collected large and medium size berries of *Diospyros mollis* were selected. Care was taken to avoid all those that were black or even streaked with black. The weight of the berries thus selected was 4,380 grams. The berries were washed, crushed in a mortar and thoroughly mixed with 1,000 milliliters of fresh coconut milk.

After a manipulation of several minutes the mixture was pressed and filtered through muslin gauze to remove the coarse particles. Coconut milk was further added to give a final volume of 3,000 milliliters. Cane sugar was then added to make the mixture more palatable. Treatment was administered immediately after preparation of the mixture thus avoiding prolonged exposure to the air. The patient was instructed to refrain from taking a morning meal prior to the administration of the drug. After vigorous stirring, 3 ml. of the medicine per year of age of the patient, up to a maximum of 75 ml., were transferred from a large container into a paper cup. After drinking the content, the patient was given warm water or tea to rinse his mouth, and was instructed not to eat for at least another three hours. No purgative was administered following treatment in view of the fact that the medicine has a laxative action. On the average, about 40 patients per hour could thus be treated. The average cost of the ingredients amounted to two cents per person treated. Administration of Maklua by age instead of body weight was the result of the following considerations: 1) In order to evaluate and to compare the results with those obtained previously in Thailand an effort was made to duplicate as closely as possible the routine of preparation and administration of the drug. 2) The above tests were carried out under field conditions where weighing of the patients was impractical.

Some patients were treated with hexylresorcinol as controls. They were instructed to eat a light evening meal and to refrain from taking their morning meal. After receiving a single dose of the drug in the amounts generally recommended, the patients were requested to return 3 hours later to receive a saline purgative. Food was not permitted for 5 hours following treatment.

RESULTS

Experiment 1. This was a preliminary experiment designed to find out the degree of hookworm reduction as measured by egg counts, brought about by a single dose of Maklua. A total of 72 people infected with hookworm, 33 males and 39 females, living in the same village, was selected for this experiment. The age range was from 5 to 61 years. Six days after treatment stools were collected for examination. Those who did not bring stools on the sixth day were asked to do so on the next day. Out of 72 treated people, five failed to submit stools for reexamination, one left the village and could not be traced afterwards, three (two males, 10 and 11 years old, respectively, and one female, 60 years old) vomited shortly after taking the medicine. They were discarded from this experimental series. The patients on the day preceding treatment were all positive for hookworm by either one of the two techniques used. The 63 included in this experimental series had an average of 1,720 hookworm eggs per milliliter of formed feces (MNE). The range was from 600 to 8,000. Six days after a single administration of Maklua, 9 patients were still positive by the Stoll, and 22 by the Willis technique. The Stoll counts in the 9 positive cases were: 800, 600, 400, 400, 200, 200, 200, 200, and 200 eggs per milliliter of feces. Assuming a value of 100 for the 13 who were Stoll negative and Willis positive, the MNE (Mean number of eggs) for the treated group was 71. In addition to the three people mentioned above who vomited, one patient had mild nausea, and two had mucous diarrhea that lasted two days. No other toxic symptoms were observed.

Experiment No. 2. In view of these results another experiment was set up to compare the effectiveness of Maklua with that of hexylresorcinol and to determine whether the egg reduction represents a true elimination or just a temporary sterilization of the worms. A total of 40 school children from the same school, 20 males and 20 females, 9 to 14 years of age was selected for this experiment. In addition to hookworm, 18 of them were infected with *Ascaris lumbricoides* and 15 with *Trichuris trichiura.*. One student treated with Maklua failed to submit his stools for examination after treatment and was discarded from the series. The 23 patients treated with hexylresorcinol, on the day preceding treatment, had an average of 4,780 hookworm eggs per ml. of formed feces. The range was from 1,000 to 15,800. The 16 patients treated with Maklua included in this series on the day preceding treatment had an average of 4,470 hookworm eggs per ml. of formed feces. The range was from 1,200 to 11,600. The results of treatment are summarized in Table 1. As measured by the Willis technique, 6 days after treatment

TABLE 1.—*Comparison of the effect of a single dose of Maklua and hexylresorcinol in hookworm infection*

Treatment	Technique	Result of stool examinations								
		Before Treatment			Six days after treatment			Ninety days after treatment		
		Number Positive	Per cent Positive	MNE[1]	Number Positive	Per cent Positive	MNE[1]	Number Positive	Per cent Positive	MNE[1]
Maklua	Stoll	16	100	4,740	0	0	31[2]	1	6	75[2]
	Willis	16	100	—	5	31	—	9	56	—
Hexylresorcinol	Stoll	23	100	4,780	7	30	187[2]	12	52	370[2]
	Willis	23	100	—	19	83	—	19	83	—

[1] MNE = Mean number of eggs per ml. of formed feces.
[2] The egg count of specimens which were Willis positive and Stoll negative was arbitrarily figured as 100 eggs per ml. of formed feces.

4 out of 23 patients treated with hexylresorcinol had no hookworm eggs in the stools. These patients were still free from infection when they were re-examined 90 days after treatment. By the Stoll technique, eggs were found in the stools of 7 people, equal to 30 per cent of the treated group. The egg counts in the 7 positive cases were 4,600, 1,200, 1,200, 1,200, 1,000, 800, and 200 eggs per ml. of formed feces. After 90 days eggs were found in the stools of 12 people, equal to 52 per cent. The egg counts in the 12 positives were 4,800, 4,200, 3,200, 600, 600, 200, 200, 200, 200, 200, 200, and 200 eggs per ml. of feces. The MNE in this group was 4,780 before treatment, 187 six days later, and 370 ninety days later.

As measured by the Willis technique, 11 out of 16 patients treated with Maklua had no hookworm eggs in the stools 6 days after treatment, and 7 of them were still free from infection when they were re-examined 90 days after treatment. By the Stoll technique, hookworm eggs were not found in the stools of any of the children treated with Maklua six days after the administration of the drug. Ninety days after treatment only one patient was positive by this method with a count of 400 eggs per milliliter of feces. The MNE of this group was 4,740 before treatment, 31 six days later, and 75 ninety days later.

It may be mentioned that negative smears were obtained 6 days after treatment in 9 out of 10 persons treated with hexylresorcinol who harbored *Ascaris lumbri-*

coides. Of these, 7 were still negative 90 days after treatment. In the group that received Maklua, negative smears were obtained 6 days after treatment in 6 out of 8 persons who harbored *Ascaris lumbricoides*. Of these, 4 were still negative 90 days after treatment. In the group which received hexylresorcinol, 7 out of 9 who harbored *Trichuris* were positive 6 days after treatment, and 8 out of 9 were positive 90 days after treatment. In the group which received Maklua, 4 out of 6 who harbored Trichuris were positive both 6 and 90 days after treatment. Two patients who received hexylresorcinol reported mild nausea. No other toxic symptoms were observed in either group.

DISCUSSION AND SUMMARY

Preliminary experiments, involving 112 patients, indicate that the green berries of *Diospyros mollis* (Maklua) crushed in coconut milk and given in a single oral administration are effective in treating human hookworm infection. Although it was not possible to recover the worms from the feces of the treated persons in view of the fact that tests were carried out in out-patient field clinics, the low egg counts 90 days after treatment suggest a true worm elimination and not just a temporary sterilization.

Diospyros mollis grows wild almost everywhere in Thailand and during the fruiting season the berries are available free or at a very low cost. It can be used in the presence of *Ascaris* infection obviating the difficulty of having the people return to the clinics for purgation after treatment. Attention is called to the fact that unless green berries are used for treatment very soon after being collected, the effectiveness is markedly reduced.

It is suggested that these preliminary results be interpreted with great caution in view of the very limited amount of work done so far on the therapeutic properties and on the toxicology of this plant. At present no contraindications have appeared. Further quantitative animal experimentation and long time observation on selected human cases are required before advocating the use of Maklua for mass treatment of hookworm in Thailand.

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OBSERVATIONS ON THE BIOLOGY OF THE LIZARD MITE *GECKOBIELLA TEXANA* (BANKS) 1904 (ACARINA: PTERYGOSOMIDAE)

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INTRODUCTION

In the course of investigating the infectivity of saurian malarial parasites to mosquitoes, 25 to 50 fence lizards, *Sceloporus undulatus undulatus* (Latreille), were maintained in the laboratory for several months. Wild-caught lizards were frequently infested with the mite *Geckobiella texana* (Banks) 1904. Acquisition of infestations by mite-free lizards and increase in density of mites on animals in captivity suggested that *G. texana* was able to propagate in the laboratory. Evidence was also obtained that the mite was responsible for transmission of a haemogregarine protozoon which occurred in wild-caught *Sceloporus*. Studies were undertaken to confirm transmission of the haemogregarine by the mite and to determine the mechanism of transmission. In pursuing these investigations the observations reported here were made on the biology of the mite. (A discussion of morphology and systematics is given in the accompanying article by Lane (1954) to whom material was referred for study.)

METHODS

The colonies used in these studies were started with the progeny of mites removed from naturally infected *S. u. undulatus* collected in Baker County, Ga. In connection with protozoa-transmission tests, it was found that *G. texana* could be maintained without difficulty for several months on the experimental lizards. Lizards were kept in one-gallon capacity glass jars provided with screened screw caps. The jars were ringed around the tops, inside and out, with vaseline to provide a barrier against escape or entrance of mites. An inch of clean sand was placed in the bottom of the jar to prevent injury to mites by the lizard.

Three different active growth stages of mites, both sexes of adults, and chrysalises of two sizes were found on the lizards. Observations were first made of stages that developed from the chrysalises; these provided information on the sequence of development of stages on the lizard. To obtain data on the number and duration of all stages, experimental mite infestations were established on mite-free lizards and development was observed at regular intervals. This was accomplished as follows: Engorged female mites removed from heavily infested lizards were isolated in ordinary glass culture tubes 75 mm. long and 12 mm. in diameter. Usually a strip of filter paper was inserted in the tube and the tubes were plugged lightly with cotton. Other types of containers with ground glass covers were tried, but they proved unsatisfactory because many larval mites were crushed on the ground surfaces. The tubes offered the advantage of permitting observation under the dissecting microscope without opening the container. Mites were removed from the tubes after ovipositing and were placed on lizards to refeed or were discarded. Ova

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were left in the tubes until hatching was complete. Larval mites were placed in a jar with mite-free lizards by unplugging the tubes containing larvae and dropping the tube and cotton plug on the surface of the sand. There were no failures to infest lizards when jars were provided with sand.

Experimentally infested lizards were examined daily for developing stages. Lizards were observed with a dissecting microscope at 10× magnification. The animals were held in a position that permitted inspection of the area beneath the scales. A fine dissecting needle was used to raise the scales and for manipulating the mites. Resting stages were removed from the lizard as soon as observed, placed on strips of filter paper, and put in the same type of tubes used for isolation of adult mites. A separate tube was used for mites removed from each lizard each day. These tubes were examined daily for ensuing stages. As soon as active forms developed they were transferred to other mite-free lizards with a long-handled camel's hair brush.

No special efforts were made to maintain constant conditions of temperature and humidity. Temperature in the laboratory varied between 22 and 29 degrees C. during the study. Jars containing lizards were heated for eight hours each day with electric light bulbs approximately one foot away. The temperature inside the jar was about 32 degrees while the lights were on; at other times the temperature approximated room temperature.

OBSERVATIONS

The following stages were observed in development of *Geckobiella texana* females: 1. egg, 2. deutovum, 3. larva, 4. nymphochrysalis, 5. nymph, 6. imagochrysalis, and 7. adult. The nymph and nymphochrysalis were omitted in development of males. The resting forms, nymphochrysalis and imagochrysalis, developed within the integument of the previous active stage. All active stages fed on blood of the host lizard. Contrary to usual behavior of ectoparasites, the mites did not leave the host soon after its death. This may have been due to the absence of a sharp temperature drop after death. The poikilothermic lizards were probably suitable hosts until the tissues became dehydrated. Mites were observed for as long as two weeks on dead lizards. Mite-free lizards placed in jars with heavily infested dead animals acquired an infestation slowly; there was not an immediate migration of mites from the dead lizards to the living lizards. Female mites that engorged on tissue fluids from the dead lizards either failed to develop ova or developed only a small number. These ova hatched in about the same proportion as those in a normal complement. Apparently blood was necessary for development of ova.

None of the engorged stages habitually left the host, except adult females which dropped from the lizard after complete engorgement. In many cases, however, females were observed to begin oviposition while still feeding. The larvae and nymphs ordinarily moved about the lizard very little but were active when removed from the host. As soon as larvae reached the lizard a place to attach was located quickly, usually under the dorsal, leg, or neck scales. Nymphs that emerged from the nymphochrysalises usually fed near the place where the larvae were attached. Often shed skins of a nymphochrysalis and of an imagochrysalis, and an engorging adult mite were observed under a single scale on the lizard. Presumably the complete development from larva to adult occurred at the site where the larva first attached.

Ovum

Ova measured 0.1 mm. × 0.2 mm., were elliptical, and appeared smooth. They were pale white, almost transparent, when first deposited, and gradually acquired a deep red color when fully embryonated. Infertile eggs remained white.

Oviposition was not observed in nature. In the lizard jars female mites oviposited on sides of the jar, grains of sand, and other surfaces. In rearing tubes eggs were placed indiscriminately on sides of the tube, on the filter paper strips, or on the cotton plugs. There apparently was no selection of surfaces. Usually a special covering for the eggs was formed by the female (Figure 1). Strands of rigid, white

FIG. 1. Oviposition by *Geckobiella texana*. Top. Ventral view of mite extending tent after partial oviposition. Center. Completed tent from below. Bottom. Completed tent from above.

fibrous material about 0.1 mm. in length were secreted singly from an orifice near the genital opening, or perhaps from the genital opening, and leaned against each other to form a tent-like structure triangular in cross section. As the strands were extruded, they were placed in position with the end of the abdomen. Usually the strands were placed successively on alternate sides of the tent but in some cases unequal numbers were placed on one side, resulting in a curved covering. One tent observed was formed in the shape of a U. The strands apparently were adhesive on the ends and remained in position without support. One end of the tent con-

sisting of eight or ten strands arranged in a semicircle was formed before the first ovum was deposited. When there was sufficient room for two or three eggs, oviposition commenced. When available space was filled the tent was extended. Note in the figure that the last ovum is some distance from the tip of the abdomen. Additional ova were probably deposited before the tent was extended.

The lengths of the completed tents varied from 1 to 8 mm.; average length of more than a hundred tents was 2 mm. The number of ova usually varied in direct proportion to the length of the tent. There were about 12 ova per millimeter of tent. Arrangement of ova within the tent was irregular. Frequently there were two irregular rows of eggs on the bottom of the tent and a third row on top of these. The maximum number of ova observed was 82 in a tent 6.5 mm. long; the usual number was about 20 to 25 in an average size tent 2 mm. long. When oviposition was completed, ends of the finished tents were usually closed with a semicircle of strands arranged in the same manner as those at the beginning of the tent.

Oviposition by a fully engorged female after the first feeding required about seven days. During this time, from three to five tents were produced, the number depending upon length of the individual tents. The maximum number of eggs observed from one female after a single engorgement was over 100. The average number of eggs per oviposition was not determined since, in most instances, some tents were formed in positions where ova could not be counted.

Deutovum

Appendage buds were prominent on the developing embryo usually about six days after oviposition. The membrane surrounding the embryo was distinctly separate from the chorion and the appearance of appendage buds was presumed to indicate the development of the deutovum stage. The egg shell ordinarily remained intact until the larva emerged but in some instances the chorion was observed to be split or torn off after development of the deutovum.

Larva

At temperatures between 22 and 29 degrees C. hatching usually occurred from 11 to 16 days, average 14 days, after oviposition. Development was accelerated at higher temperatures and prolonged at lower temperatures. Hatching of a lot of ova usually was completed within a day or two, and no ova were observed to hatch later than five days after hatching of the first egg in a lot. The period of embryonic development apparently was constant; hatching over a period of several days was due to length of time required for oviposition of a lot of eggs.

The larva emerged through a lateral slit that extended about half the length of the egg. The small six-legged larvae were very active. In rearing tubes, larvae moved constantly over sides of the tube and congregated at the cotton plug.

Feeding was not observed until the third or fourth day after hatching. The first nymphochrysalis was observed eight or nine days after hatching. Allowing a four-day period before the larvae fed, engorgement required a minimum of four or five days. The average time required for engorgement was longer, however. The maximum number of nymphochrysalises was removed from the infested lizard on the sixth or seventh day after the first nymphochrysalis was observed. After this, 14 or 15 days after mites were placed on the lizards, there was a decline in the number of nymphochrysalises that developed daily. Active, unengorged larval

mites were observed as long as 30 days after mites were placed in jars; nymphochrysalises were removed up to 35 days. Larval mites confined in rearing tubes were able to survive for 21 to 40 days without feeding.

Nymphochrysalis and Imagochrysalis

After larvae were fully engorged the nymphochrysalis developed in the larval integument. In the initial stage nymphochrysalises were difficult to distinguish from a quiescent engorged larva. Complete engorgement of larva was necessary for development of the nymphochrysalis. Removal of the larva before complete engorgement invariably caused death of the mite before development of the nymphochrysalis.

Mites in the resting stage following larval engorgement were of two sizes. The larger ones measured approximately 0.5 mm. long and 0.25 mm. wide; the smaller ones about 0.4 mm. long and 0.2 mm. wide. Little overlap was observed. The two sizes could be distinguished with considerable accuracy without measurement. By separating the sizes it was determined that only adult male mites emerged from the larger size and only female nymphs emerged from the smaller size. Following the twentieth post-hatching day, large forms were more than twice as abundant as the small ones. Nymphs usually emerged from the nymphochrysalises in four to seven days. Males usually emerged between the seventh and tenth days.

Development of the imagochrysalis of the female required from four to seven days and was similar to the nymphochrysalis except for its larger size.

Nymph

The nymph emerged from the nymphochrysalis through an anterior lateral slit that extended about half the length of the body. The front pair of legs was projected first and the nymph quickly emerged. In some instances the slit encircled the body completely, dividing it into two halves. The nymphs were superficially similar to adult females but could be distinguished from them readily by the length of the first pair of legs; those of the nymph were approximately 0.35 mm. long as compared with a length of 0.6 mm. in the adult female.

Nymphs were active and became attached to the host soon after emerging. They were unable to survive longer than three or four days without feeding. Engorgement of the nymphs required from seven to fifteen days. When fully engorged, the nymphs became quiescent and the imagochrysalis was formed inside of the nymphal integument in the same manner as the nymphochrysalis formed within the larval integument.

Adult (Female)

The adult female emerged from the imagochrysalis in the same manner that the nymhp emerged from the nymphochrysalis. The newly emerged adults were very active and began feeding immediately if placed on a lizard. Engorgement was slow; seven to ten days were required for the adults to feed to repletion. Fully engorged females removed from lizards usually began oviposition within a day after being placed in a rearing tube. It was not possible to determine longevity or the number of successive feedings by observing individual mites. Some female mites fed three times and deposited three successive groups of eggs over a period of 25 days. The life span probably is much longer than this. Females survived for 17 days without

feeding, although most died by the tenth day. After completion of oviposition the adult died if another feeding was not obtained within a few days.

Mating was not observed but probably occurred prior to feeding. Ova in successive lots obtained from females that refed hatched in the same proportion as did the initial lot.

Parthenogenetic development was observed. Female mites that were not given an opportunity to mate produced comparatively small complements of fertile eggs. Fertility was low but all adults (38) reared from such ova were males.

(*Male*)

The most striking feature of the male was the shape of the first pair of legs. Because of the smaller body of the male they appeared much longer than the front legs of the female, although they were approximately the same length. Unlike those of the female, the legs were curved outward to form hook-like appendages that appeared to be modified for grasping or holding.

Males attached to the host soon after emergence from the imagochrysalis. Some individuals survived for 14 days without feeding. Males took small blood meals and refed frequently; they did not become greatly distended. Males were quite active and detached themselves from the host readily. The exact length of life is not known but males were observed to live for 20 days; average life was probably longer.

SUMMARY

The biology of a pterygosomid mite, *Geckobiella texana* (Banks) 1904, was studied in connection with investigations of the transmission of a saurian haemogregarine in the fence lizard, *Sceloporus undulatus undulatus* (Latreille). Colonies of mites were maintained on lizards in the laboratory. Larvae, nymphs, and adult male and female mites fed on blood. Oviposition in nature was not observed, but in the laboratory no preference was shown among the oviposition sites available. Development of the female, from egg to gravid adult, required from 42 to 86 days. The stages recognized and the time of development at temperatures between 22 and 29 degrees C. were as follows: 1. egg, 6 to 8 days; 2. deutovum, 5 to 8 days; 3. larva, 9 to 31 days; 4. nymphochrysalis, 4 to 7 days; 5. nymph, 7 to 15 days; 6. imagochrysalis, 4 to 7 days; 7. adult female, 7 to 10 days for engorgement. The male omitted the nymph and nymphochrysalis stages. The resting stages developed within the integument of the succeeding active stage.

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THE LIFE HISTORY OF *NEMATODIRUS HELVETIANUS* MAY, 1920, A NEMATODE PARASITIC IN CATTLE[1]

HARRY HERLICH

INTRODUCTION

Nematodirus helvetianus May, 1920, commonly known as the thread-necked strongyle, is a trichostrongylid nematode found in the small intestine of cattle and occasionally also of sheep, goats and camels. Inasmuch as Herlich and Porter (1953) found that this strongyle is potentially pathogenic to calves, it was considered desirable to determine its life history.

The only previous work dealing with the life history of *N. helvetianus* was done by Zviagintsev (1934) who reported that larval development and molting occurred within the egg. Observations made on the effects of specific temperatures on the rate of development will be discussed in conjunction with results of this investigation. Zviagintsev also noted that the infective larvae were very resistant to desiccation, and could live as long as eight months or more at room temperature. He was unable to infect guinea pigs or rabbits, but succeeded in infecting a lamb. He found that larvae fed to calves reached maturity in three to four weeks.

Nearly two decades earlier Boulenger (1915) studied the life history of a closely related species, *N. filicollis* (Rudolphi, 1802) Ransom, 1907, a parasite of the small intestine of sheep. He reported that the embryo developed within the egg and underwent two molts, one complete and the second incomplete. This confirmed earlier observations by Ransom (1911), Railliet and Henry (1912) and Maupas and Seurat (1913). Since the infective larva retained the cuticle of the second molt, Boulenger considered it to be in a state of transition between the second and third stage. He noted that the infective larva shed its sheath *in vitro,* when exposed to temperatures approximating the body temperatures of mammals. Parasitic larval stages were described by Boulenger on the basis of immature specimens recovered from naturally infected sheep killed for post-mortem examination. He found the first traces of external sex characters in specimens 2.5 mm. long. In the male, this appeared as a distinct swelling just anterior to the tail fork, at about the level of the anal opening. This he considered to be a rudimentary bursa. Specimens measuring 5 mm. long exhibited bursal rays and spicules, and at this stage of development, the characteristic tail fork and caudal process had disappeared. In the young female, the tail fork and caudal process were retained somewhat longer, the process being retained as the terminal spike characteristic of the mature female.

MATERIALS AND METHODS

The initial supply of infective larvae was cultured from eggs passed in the feces of a calf naturally infected with *N. helvetianus* only. Since attempts to culture eggs in feces-charcoal and feces-sphagnum-moss (Cauthen, 1940) mixtures were unsuccessful, it was necessary to recover the eggs from the manure. Kauzal's (1939)

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technic for recovery of eggs of the genus *Nematodirus* failed to yield a satisfactory harvest; therefore, the following technic of sedimentation and flotation was devised. Feces were thoroughly comminuted and washed through a sieve (22 mesh to the inch) into large battery jars, where the filtrate was allowed to settle overnight. The supernatant fluid was poured off and discarded. The sediment, containing the eggs, was mixed with a supersaturated solution of common salt (sp.gr. 1.2), poured into 250 cc. bottles and centrifuged at 1500 r.p.m. for one to two minutes. The top 10 cc. were then siphoned off, added to water and recentrifuged. Following this centrifugation, the supernatant fluid was siphoned off and discarded, leaving a large number of eggs concentrated in approximately 20 cc. of water.

Eggs were cultured in petri dishes containing tap water to a depth of 2 mm. A few drops of 2 per cent formalin were added to each dish to retard bacterial contamination. Since the embryos complete their development within the egg, hanging drop preparations of individual eggs were observed to determine the exact period of molting.

Measurements were made on eggs freshly recovered from feces, and all measurements of the preparasitic and parasitic stages were made on heat-killed unstained specimens floating freely under the coverslip to prevent distortion. Drawings were made with aid of a micro-projector.

Calves used in this study were parasite-free, grade Jerseys purchased from local dairies within 24 hours of birth. They were raised in portable pens (Davis, 1949) until used for infection experiments, at which time they were moved to individual stalls in a concrete-floored barn, and maintained in the manner described by Porter and Cauthen (1942).

Infective larvae in water were fed to calves by means of a blowpipe attached to a 5 cc. rubber bulb. The number of larvae fed was determined by dilution count, except when the number was low enough to permit a direct count. The course of infection of each calf was followed by fecal egg counts and is expressed as eggs per gram (EPG), using the technic described by Stoll (1930).

Immature parasitic stages were recovered from calves killed five hours, 4, 8 and 15 days after infection. Pieces of tissue from various levels of the upper small intestine were removed and fixed in 10 per cent formalin for sectioning. Routine post-mortem examinations at the Laboratory indicated that the greatest number of *N. helvetianus* were located in the first fifteen feet of the small intestine.

RESULTS

Preparasitic Stages

Egg: All measurements in microns. Eggs recovered from fresh fecal material were in the 2-cell to 8-cell stage of development, the 8-cell stage being observed most often. The eggs which are larger than those of any other gastrointestinal nematode of cattle are ovoid and clear, with the cell mass centralized (Plate I-A). May (1920) found that the eggs were 160–230 long and 85–110 wide. Shorb's (1939) measurement of preserved eggs showed them to be 184–233 long by 84–110 wide, the mean being 212 by 96.7. In this study, 100 eggs, recovered from fresh feces, ranged from 185–245 by 92–113, the mean being 208 by 106. Standard deviation for length was 15; for width 3.

Temperature effect on embryonic development: Zviagintsev (1934) reported that the eggs are very resistant to cold, withstanding Moscow frosts at different

stages of development. He found that they develop to infectivity within a range of 3° to 29° C., the optimum temperature being 28° C. At higher temperatures, he noted that 50 per cent of the eggs died when cultured at 34° to 35° C. and that all the eggs perished at early stages up to the morula when cultured at 38° to 39° C. In the present study, eggs which were still in the 8-cell stage after being kept at −10° C. for 21 days proceeded to develop normally when placed in an incubator held at 28° C. Eggs kept at 3° C. for one week failed to develop beyond the 16-cell stage. When placed in the incubator held at 28° C., normal development took place. When cultured at 34° to 35° C. for 10 days, about 65 per cent of the eggs contained viable larvae which were liberated five days later. The remaining 35 per cent of the eggs ceased developing at stages varying from the 8-cell to the tadpole stage. Although most of the eggs cultured at 38° to 39° C. perished during the early stages of development, about 15 per cent succeeded in reaching the infective stage. At room temperatures ranging from 20° to 22° C., approximately 75 per cent of the eggs developed to the infective stage. Hatching began 17 to 20 days after incubation and continued sporadically for as long as 121 days after incubation. Optimum temperature for development was 28° to 29° C. with 85 to 90 per cent of the eggs reaching the infective stage. At this temperature, hatching commenced

TABLE 1.—*Development of eggs of* Nematodirus helvetianus *after incubation at 28° to 29° C.*

Hours After Incubation	Stage of Development
0	8 cells
16	32 to 64 cells
24	Morula
40	Blastula and gastrula
48	Tadpole
64	First-stage larva
93	First-stage larva in lethargus
96	Second-stage larva
144	Second-stage larva in lethargus
160	Third-stage larva
192	Hatching commences

on the eighth to the tenth day and nearly all the eggs completed hatching by the eighteenth to the twentieth day. The course of development of the egg to the infective stage at optimum temperatures is outlined in Table 1.

First-stage larva: First-stage larvae, moving very sluggishly within the egg, were observed after 64 hours' incubation at 28° C. The larva was filled with disorganized, granular material, except for a clear spot in the cephalic region, the primitive buccal cavity. Measurement of early first-stage larvae was impossible because they disintegrated when liberated from eggs ruptured mechanically. Just prior to the first ecdysis, the granular material became denser in the area destined to become the intestinal tract. Ten larvae measured at this stage of development showed an average length of 990 microns (Plate I-B).

Second stage larva: Towards the end of the fourth day of incubation (93 hours), the larva became lethargic, and three hours later it commenced writhing actively. This was the second-stage larva enclosed in a loosely fitting sheath, actually the cuticle of the first-stage larva (Plate I-C). At this stage, the genital primordium was seen as a lenticular-shaped body. The caudal as well as the cephalic area was clear. The intestinal tract was becoming differentiated into a definite cellular wall; however, the esophageal region was still obscured by dense granular material. Measurement of 10 second-stage larvae just before the onset of the second molt

showed an average length of 1075 microns, this representing a growth of 85 microns over that of the first-stage larva. The genital primordium consisted of numerous small cells, and was about 18 microns long by 10 microns wide.

By the end of the sixth day of incubation the second-stage larva ceased its active movement. The second molt began at this time, and was completed sixteen hours later. The egg shell contained a larva enclosed in two sheaths, the outer one fitting loosely, and the inner one rather tightly. The latter could be seen as a fine plicate membrane in those regions where the larva was coiled on itself (Plate I-D).

Hatching began on the eighth to tenth day of incubation, and practically all the larvae were liberated by the end of 20 days. As the larva was wriggling out of the egg, the outermost sheath (the cuticle of the first-stage larva) was stripped off (Plate I-E) and it could readily be seen inside the eggshell, along with the vitelline membrane, after the larva had completely emerged (Plate I-F). The liberated larva was in the infective third stage (Plate I-G), still being ensheathed within the cuticle of the second stage. The sheath was most readily seen at the extremities. Measurement of 100 third-stage larvae gave an average total length, including the sheath, of 1106 microns and an average width, at the widest point, of 35 microns. At the anterior end there were no distinct lips, but there were six

TABLE 2.—*Measurements in microns of 100 infective larvae of* Nematodirus helvetianus *and corresponding measurements of* N. spathiger *and* N. filicollis

	N. spathiger Dikmans and Andrews (1933)	*N. filicollis* Boulenger (1915)	*N. helvetianus* Present Writer	S.D.
Total length including sheath	922–1118 (1009)*	850–1150 (1000)	974–1248 (1107)	62
Length of esophagus	160– 225 (195)	180– 220 —	191– 252 (226)	14
Anterior end to genital primordium	365– 500 (417)	480 —	363– 568 (488)	44
Length of tail	52– 65 (60)	50 —	51– 69 (62)	5
Anus to posterior tip of sheath	315– 350 (328)	300 —	269– 368 (321)	21

* Figures in parentheses represent means. S.D. Standard Deviation.

small refractive areas which were the rudimentary cephalic papillae. The buccal cavity led into an esophagus which still remained somewhat obscured by the granular material surrounding it. The intestinal tract was composed of eight alternate cells, four of which were dorsal and four ventral. These cells were filled with granular material, making it somewhat difficult to see the nuclei. A slender rectum led from the intestine to the anus, which opened ventrally and was subterminal. The genital primordium was located about two-thirds the body length from the anterior end, and it had the same dimensions as those noted in the second-stage larva, but there was a noticeable increase in the number of cells. The nerve ring could be seen about 140 microns from the anterior end, or just cephalad to the excretory pore, the latter opening ventrally. The tail had a dorsal and ventral lobe between which lay a rod-shaped process extending about 15 microns beyond the posterior margin of the lobes. The infective larvae were very active in water, swimming with a whip-like motion. They were readily differentiated from the larvae of other cattle nematode species by the forked tail and median rod, and also by the extreme attenuation of the sheath at the caudal extremity. The measurements of 100 infective larvae are summarized in Table 2 along with the corresponding measurements of the infective larvae of *N. spathiger* and *N. filicollis* as recorded by Dikmans and Andrews (1933) and by Boulenger (1915).

The infective larvae of these three species closely resemble one another. However, the larva of *N. helvetianus* has an over-all length averaging about 100 microns longer than that of either of the other two species. In addition, Andrews (1935) described the buccal cavity of the infective larva of *N. spathiger* as having an inverted globular shape (Plate I-I). The present study showed that the shape of the buccal cavity of the infective larva of *N. helvetianus* is distinctly hexagonous (Plate I-H).

Parasitic Stages

Third-stage larva: Ensheathed larvae, comparable in size and structure to the third-stage infective larvae, were recovered from the omasum and abomasum of a calf which was killed and examined five hours after being fed 26,500 infective larvae of *N. helvetianus.* No exsheathed larvae were seen, indicating that the second molt is probably completed in the small intestine.

A second calf was killed four days after having been fed 200,000 infective larvae in a single dose; exsheathed larvae were recovered from the lumen of the small intestine. These were the parasitic third-stage larvae, ten of which averaged 1180 microns in length and 39 microns in width, making them only slightly longer and wider than the original infective larvae. Morphologically, they differed from the preparasitic third stage in that the intestinal tract had transformed from a simple eight-celled structure into a complex multicellular tract. The genital primordium still appeared as a lenticular-shaped body, about two-thirds from the anterior end of the larva. The genital primordium consisted of numerous cells, and measured about 28 microns long and 11 microns wide. The exsheathed larvae still retained the characteristic tail structure of the infective stage, namely, the two lobes with the rod-shaped process lying between and extending beyond them (Plate II-A). Histological sections failed to reveal any evidence of larval penetration of the intestinal wall of the calf.

Fourth-stage larva: Ensheathed larvae were recovered from the lumen of the small intestine of a calf killed eight days after it had been fed 80,000 infective larvae. These were fourth-stage larvae in which the sexes were readily distinguishable, the females being somewhat longer and wider than the males. Ten males averaged 3.5 mm. in length and, except for the area anterior to the anal opening, were uniformly 48 microns in width, tapering at the cephalic and caudal extremities. At the level just anterior to the anal opening, the body had increased to a width of 58 microns, this dilatation being the rudimentary bursa (Plate II-D). Internally, there were no distinct sex characters. Ten females averaged 4.3 mm. in length by 64 microns in width, except at the tapering extremities. In contrast to that of the males, the area just anterior to the anal opening averaged only 38 microns in these ten females. About 0.9 mm. from the tip of the terminal spine there was a clearly formed vulva and rudimentary ovijectors (Plate II-C). The total length of the ovijectors in ten females averaged 238 microns. In both the males and females, the heads were 21 microns wide and bore six cephalic papillae (Plate II-B). Both the males (Plate II-D) and females (Plate II-E) had lost their forked-tail structure, but both sexes still retained the rod-like process in the form of a finely attenuated terminal spine. At this stage there was no evidence of larval penetration of the intestinal wall.

Fifth stage or young adults: Eighty-seven thousand infective larvae were fed

to a calf, which was killed 15 days later. Worms were recovered from the lumen of the small intestine. Fourteen per cent of the nematodes so recovered were still fourth-stage larvae. The remaining 86 per cent were fifth-stage worms, with approximately 12 per cent still undergoing the final molt. Young fifth-stage worms resembled mature adults in all respects, except in size and sexual development. Ten males averaged 5.4 mm. long, and had fully-formed bursae in which the rays were distinct. At its widest point, the bursa averaged 140 microns in diameter in the ten specimens aforementioned. The spicules were lightly sclerotized and measured about 400 microns in length, or nearly half as long as those of the adult worm. Ten females which had an average length of 6.4 mm. were non-gravid. The posterior end of the fifth-stage female (Plate II-F) was truncated and terminated in a spine which is a distinguishing characteristic of the adult females of the genus *Nematodirus*. At this stage of development the young nematodes were in intimate contact with the intestinal wall, being coiled among the villi. There was degeneration of the epithelium of some of the villi adjacent to the worms; a catarrhal exudate was present. The latter was probably the result of mechanical irritation produced by the friction of the worms against the villi. It is interesting to note that Herlich and Porter (1953) found that *N. helvetianus* exerted its most detrimental effects during the prepatent period, and that clinical symptoms of parasitism, including diarrhea, anorexia and weakness, were shown by two calves 15 days after they had been fed massive numbers of infective larvae.

Mature adults: Ninety-eight adult nematodes were recovered from the small intestine of a calf killed 79 days after having been fed 560 infective larvae. This represented 17.5 per cent of the original number of larvae fed. Eighty-two were females and 16 were males. On the basis of length and shape of the spicules and width of the membranous expansion at the tip of the spicules, a specific diagnosis of *N. helvetianus* was made. The nematodes conformed in all respects with the descriptions given by May (1920) and Travassos (1937). The males were from 11–17 mm. long and 150–200 microns wide at the broadest point. The spicules were from 0.9–1.25 mm. long and the terminal membranous expansion was from 19–22 microns wide. The females were 18–25 mm. long, with the vulva located about two-thirds from the anterior end. For a complete description of the adults, the reader is referred to the classic descriptions of the authors cited above.

Course of Infection

Prepatent period: The time interval between exposure to infective larvae and the appearance of eggs in the feces of the host was determined in nine experimentally-infected calves. Table 3, which summarizes the data pertinent to these infections, shows that the prepatent period ranged from 21 to 26 days, with more calves passing eggs in the feces 21 days after infection than at any other time. There was no apparent relationship between prepatent period and age of the animal at time of infection.

Patent period: Table 3 shows that the patent period ranged from 12 to 132 days in six of the experimentally infected calves. A seventh calf (1129), necropsied 79 days after infection, was still passing eggs in the feces just prior to death. Another calf (1199) was passing eggs in the feces when transferred to a contaminated lot 94 days after infection. The patent period of Calf 1205 could not be determined,

as this animal was exposed to a second dose of infective larvae in an attempt to produce superinfection. It is of interest to note that the longest patent period was observed in Calf 1255, the youngest of the test animals in which the patent period could be definitely determined. On the other hand, the infection of the oldest animal, Calf 1193, resulted in an extremely short patent period of only 12 days. These two calves, with extreme patent periods, had been fed comparably moderate numbers of infective larvae.

Egg production: Table 3 shows that in nine experimental infections the maximum fecal egg count ranged from 6 to 1,164 EPG. Very low counts were recorded for all calves that had been fed only a few hundred larvae. Paradoxically, a peak only slightly higher than the lowest aforementioned was observed in the infection of Calf 1203, which received 1,000,000 infective larvae. It is of further interest to

TABLE 3.—*Data on experimental infections with* Nematodirus helvetianus *in 9 calves*

Calf Number	Date of Birth	Infective larvae fed		Prepatent period (days)	Patent period (days)	EPG	
		(date)	(number)			(max. number)	(days after infection)
1122	5–18–1949	10–19–49	620	21	115	8	28
1255	11–15–1951	2–12–52	28,000	21	132	144	125
1187	11– 5–1949	6–23–50	32,000	24	66	120	44
		6–24–50	75,000				
		6–25–50	33,000				
		Total	140,000				
1205	8– 8–1950	9– 5–50	44,000	23	--	390	35
		9– 6–50	50,000				
		9– 7–50	56,000				
		Total	150,000				
		11– 1–50	142,000	—	—	1164	28[c]
		11– 2–50	135,000				
		Total	277,000				
		2– 9–51	100,000				
1203	7–17–1950	1– 2–51	1,000,000	21	111	14	35
1193	1– 4–1950	10–25–50	16,000	26	12	6	38
1129	6–17–1949	12– 9–49	560	26	79[a]	10	52
1159	8–11–1949	12–28–49	371	26	93	10	28
1199	6–23–1950	2– 9–51	100,000	21	94[b]	320	32

[a] Eggs being passed when killed.
[b] Eggs being passed at last observation possible.
[c] Days after challenge dose.

note that the highest peak of egg production occurred during the period of superinfection of Calf 1205. On the basis of fecal egg counts, it appears that a single dose of 28,000 to 100,000 infective larvae resulted in development of the greatest numbers of adult worms.

Superinfection and reinfection: As is shown in Table 3, Calf 1205 was infected at one month of age by feeding 150,000 infective larvae, divided in three daily doses. This calf passed eggs in the feces 23 days later, and the fecal egg count reached a peak of 390 EPG about one week later. Four weeks after the first passage of eggs, the count had dropped to only a few EPG. When the calf was three months old and still passing eggs, it was fed 277,000 infective larvae in two daily doses to determine whether it was resistant to superinfection. Three weeks after the challenge dose, the fecal egg count began to rise rapidly, finally reaching a peak of 1,164 EPG. It is concluded from these data that superinfection was successfully produced in this calf. The fecal egg count fell rapidly from the peak observed during the period of superinfection, and nine weeks after the calf had received the challenge dose, eggs could not be found in the feces. After two weeks with no eggs

in the feces, a second challenge dose of 100,000 infective larvae was given, to determine whether the calf was still resistant to reinfection. No eggs were found in fecal examinations made during the following two months.

SUMMARY AND CONCLUSIONS

1. The life history of *Nematodirus helvetianus* is direct.

2. The eggs are clear, ovoid, larger than those of any other nematode parasite of cattle, and usually in the 8-cell stage when passed in the feces. The eggs are extremely resistant to cold, being able to develop normally after 21 days' exposure to – 10° C.

3. Development to the infective stage occurs within the egg, the larva undergoing two molts and retaining the cuticles of both the first and second stage as sheaths, until it hatches.

4. At the optimum temperature of 28° to 29° C., the larva may hatch after 8 to 10 days incubation. At the time of hatching, the cuticle of the first stage is shed by the escaping larva.

5. The infective larva is longer than that of any other nematode parasitizing cattle. The mean length, including the sheath, is 1,106 microns. The tail is forked, and between the lobes there is a rod shaped process extending beyond the posterior margins of the lobes.

6. When the infective larva is ingested by a susceptible calf, it completes the second molt in the small intestine sometime prior to the fourth day of infection. The third-stage larva in the host is easily distinguished by the forked tail and median rod shaped process.

7. The larva is in the fourth stage by the eighth day of infection, at which time it is found free in the lumen of the small intestine. Sex is readily differentiated at this stage; the male has a rudimentary bursa, which is seen as a marked dilatation of the body just anterior to the anal opening. In the female the vulva and rudimentary ovijectors are present. The most distinguishing characteristic of the fourth-stage larva is the loss of the forked tail and the retention of the rod-shaped process as a terminal spine, in both sexes.

8. By the fifteenth day of infection most of the nematodes are fifth-stage worms resembling the adult stages, except in size and degree of sexual development. The worms are in close contact with the intestinal wall, coiled around and among the villi of the small intestine.

9. Extensive penetration of the intestinal wall by the early parasitic stages was not noted in these studies. Catarrhal inflammation, and some destruction of villi, were observed in a calf harboring young fifth-stage worms.

10. The prepatent period in experimental infections of calves, 1–10 months old, ranged from 21 to 26 days. There was no correlation between length of prepatent period and age of the calf host at time of infection.

11. The patent period in eight experimental infections ranged from 12 to 132 days. There appeared to be some correlation between the patent period and the age of the calf at time of infection, the oldest calf having shown the shortest and the youngest calf the longest patent period observed.

12. On the basis of fecal egg counts, administration of 28,000 to 100,000 infective larvae resulted in the development of the greatest numbers of adult nematodes.

13. Superinfection was successfully established in a three-month-old calf which

had originally been infected experimentally at one month of age. This calf was resistant to reinfection at six months of age.

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Explanation of Plates

Plate I

(Drawn with the aid of a micro-projector)

Fig. A. Egg from fresh feces.
Fig. B. Egg containing the first-stage larva.
Fig. C. Egg containing the second-stage larva.
Fig. D. Egg containing the third-stage infective larva.
Fig. E. Third-stage infective larva emerging from the egg.
Fig. F. Hatched egg containing the vitelline membrane and the cuticle of the first molt.
Fig. G. Third-stage infective larva, lateral view.
Fig. H. Anterior end of the third-stage infective larva of *Nematodirus helvetianus.*
Fig. I. Anterior end of the third-stage infective larva of *Nematodirus spathiger* (after Andrews, 1935).

Plate II

(Drawn with the aid of a micro-projector)

Fig. A. Posterior end of the parasitic third-stage larva, lateral view.
Fig. B. Anterior end of fourth-stage larva, lateral view.
Fig. C. Vulva and rudimentary ovijectors of a female fourth-stage larva, lateral view.
Fig. D. Posterior end of a male fourth-stage larva, lateral view.
Fig. E. Posterior end of a female fourth-stage larva, lateral view.
Fig. F. Posterior end of a female fifth-stage nematode, lateral view.
Fig. G. Posterior end of a male fifth-stage nematode, dorso-ventral view.

PLATE I

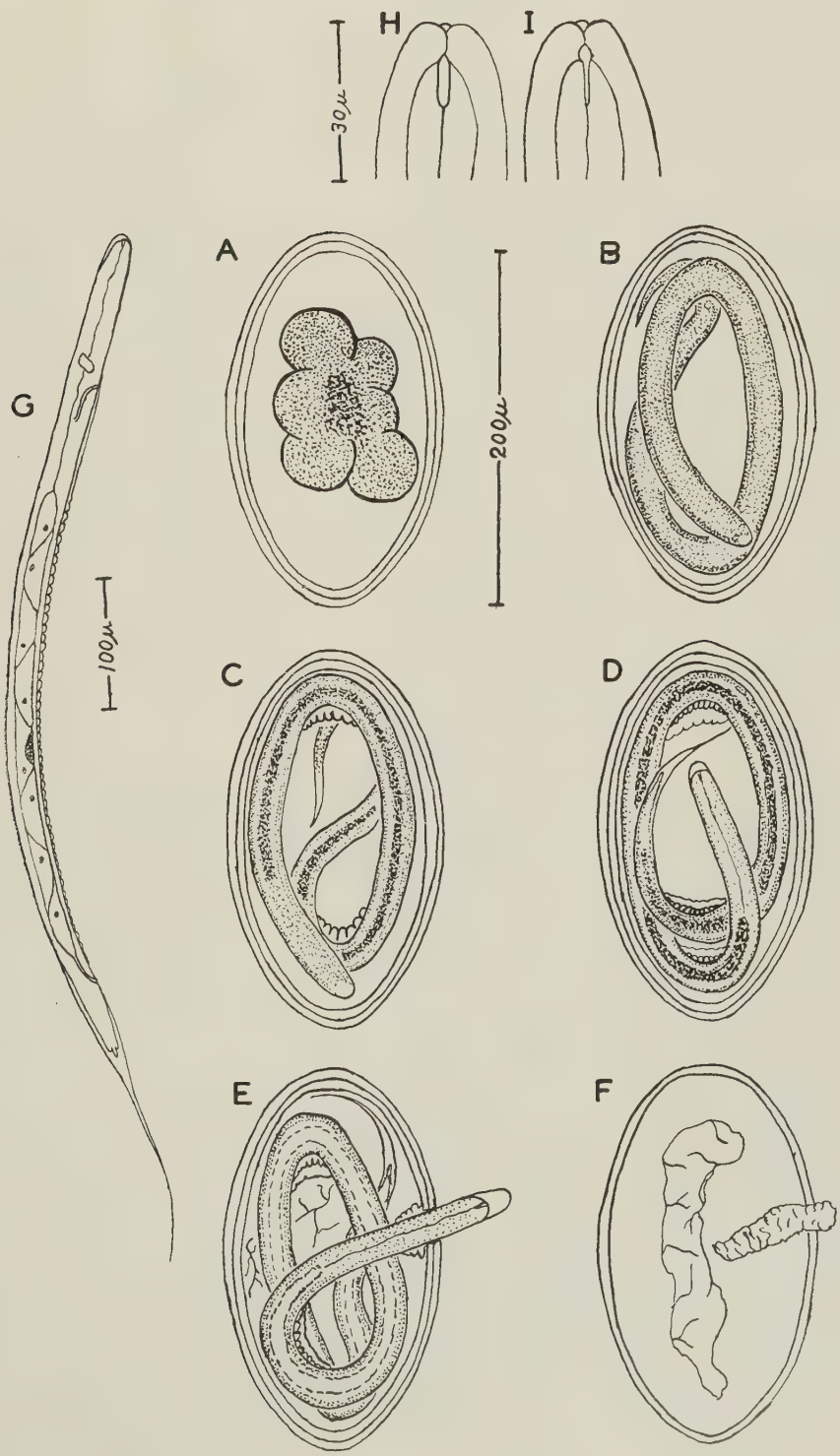
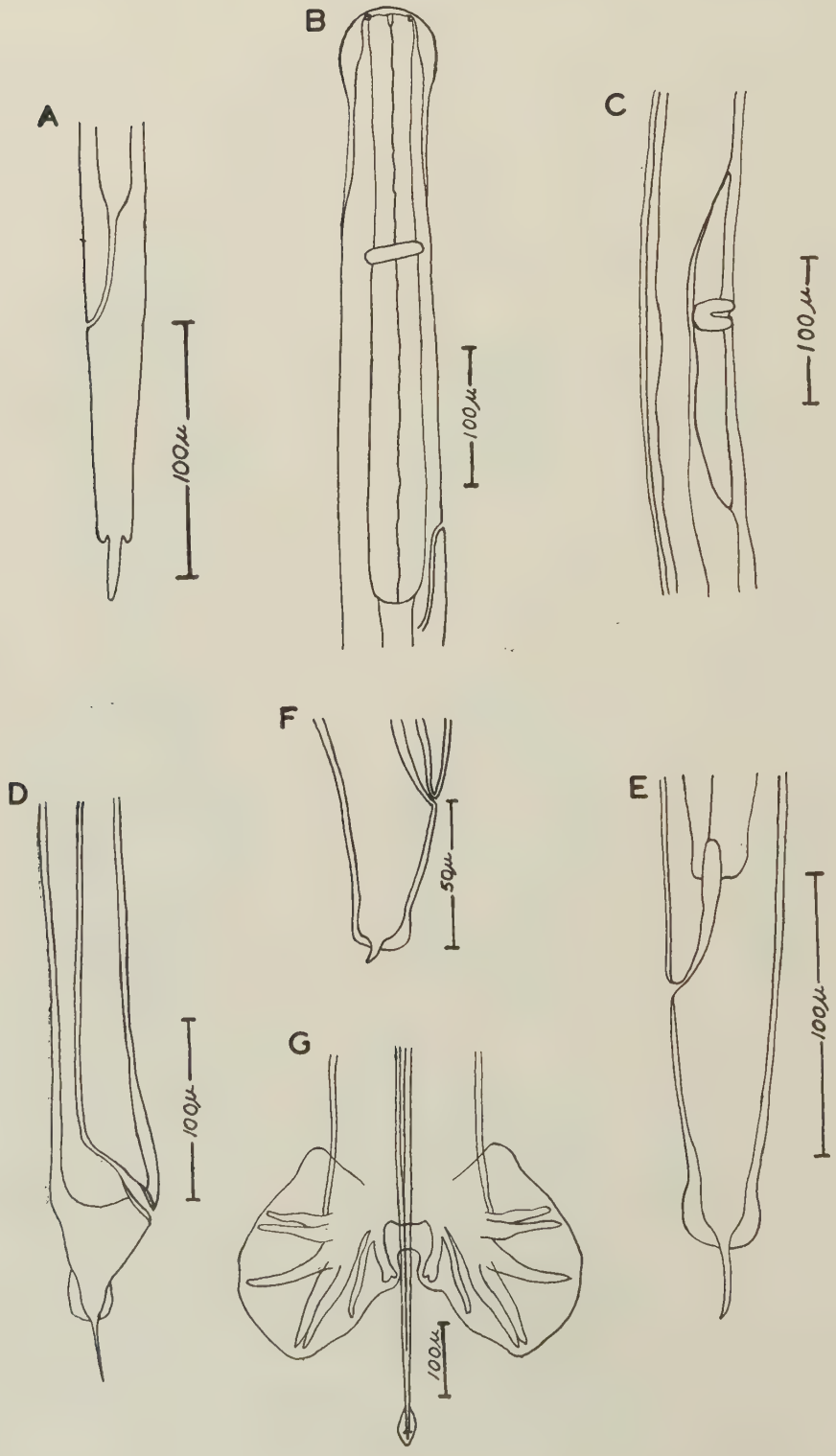


PLATE II



STUDIES ON MARINE DIGENETIC TREMATODES OF PUERTO RICO. THE LIFE CYCLE IN THE FAMILY HAPLOSPLANCHNIDAE

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INTRODUCTION

The family Haplosplanchnidae was erected by Poche (1926) to include the single genus and species, *Haplosplanchnus pachysomus*. Other trematodes were not assigned to the family until Manter (1937) showed that the genus *Deradena* Linton was in part synonymous with *Haplosplanchnus* Looss and transferred Linton's *D. obtusa* and *D. acuta* to *Haplosplanchnus*. Manter also redescribed these species and reported four additional ones, *H. sparisomae, H. brachyurus, H. pomacentri* and *H. adacutus,* all from Tortugas fishes. Srivastava (1939) added *H. purii* and a new genus and species, *Laruea caudatum,* to the family. Manter (1940) reported *H. acutus* and *H. pomacentri* from Galapagos fishes and later (1947) described *H. kyphosi* from chubs at Tortugas.

The family Haplosplanchnidae thus is one of the smaller groups of digenetic trematodes and is restricted to marine fishes that are largely herbivorous. At least two and perhaps more species of *Haplosplanchnus* were found by the writer in Puerto Rico; the exact number will not be known until the collection is studied further. To date, there has been no account of a life cycle in the family Haplosplanchnidae except the abstract of the present study (Cable, 1952).

METHODS

The information presented in this paper was obtained during the survey and study of as many marine larval and adult trematodes as possible during an eleven-month investigation in Puerto Rico. The cercaria reported here develops in *Cerithium variabile*, an extremely abundant snail which was collected in large numbers with a scraper net. The usual methods of isolation for infections and studying cercariae were used. Figures 1–5 were drawn free-hand and scale drawings are based on numerous measurements, those of the cercaria being taken from specimens handled in the following manner. Several freshly emerged and actively swimming larvae were transferred to a small amount of water in a stender dish, and a large volume of sea water heated to boiling was poured into the dish. The larvae thus killed by heat settled quickly to the bottom and were transferred to a slide with sufficient water that no pressure whatsoever was exerted on them when a cover glass was added and measurements were taken. This method has the advantages that it can be duplicated by any one, is rapid, and eliminates the sources of error inherent in attempts to measure living cercariae or fixed and stained specimens. It does render certain types of cercariae so opaque as to cause difficulty in measuring internal structures, no more so, however, than does hot formalin or fixing solutions sometimes employed. With most of the numerous species of

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cercariae handled in this manner, there was very little variation in size. When haplosplanchnid cercariae were heat-killed, however, evidently the abortive attempt to encyst resulted in rather more than the usual extremes of body length.

OBSERVATIONS

Evidence Bearing on the Life Cycle in the HAPLOSPLANCHNIDAE.

Neither time nor adequate facilities were at the writer's disposal to utilize experimental procedures essential to a demonstration of the life history beyond question. The evidence to be presented therefore is circumstantial and based on the morphology and ecology of what are believed to be stages in the life cycle of a species of *Haplosplanchnus*.

All material mentioned in this connection was collected from a small area of Salinas Bay at Cabo Rojo, one of the most productive of the habitats found. An intensive examination of both mollusks and fishes from that area was made and several birds were collected from a nearby mud flat. Water in the bay was shallow and a seine could be used for a considerable distance from shore. Furthermore, a fisherman had twelve traps in the deeper water and examples of all the fishes taken in them over a period of several weeks were obtained and examined. Thus many adult and larval trematodes were available from a rather circumscribed habitat which in its biota was far more constant than might be supposed.

Among the cercariae found in that area, and incidentally nowhere else although its host was extremely abundant, was the species described below. After swimming for a time, it encysted in the dish in an entirely normal manner and not abortively as some cercariae tend to do after failing to find a second intermediate host. This suggested the possibility of the absence of such a host in the life cycle.

Several features of the cercaria provided significant clues to the adult and it soon became apparent that of all the trematodes that had been found in the habitat only some member of the family HAPLOSPLANCHNIDAE could qualify for that rôle. Perhaps the most striking and suggestive character was the rhabdocoele intestine which was so well developed in the cercaria that it could hardly be considered to develop later in a bifid gut. Since a rhabdocoele intestine is decidedly rare in distome groups as a whole, it seemed that all except the HAPLOSPLANCHNIDAE could be ruled out on the basis of the digestive system alone. Furthermore, adults of that family contained remnants of cercarial eye-spots and much body pigment as did the cercaria, both lacked cuticular spines, and they agreed in respect to the excretory system. Also, the structure of the oral sucker of the cercaria with the characteristic papilla on the ventral lip was the same in adult haplosplanchnids that had been found. Finally, encystment in the open and a tendency of the cysts to adhere to vegetation suggested that the definitive host would feed on vegetation and adult haplosplanchnids occur predominantly in herbivorous fishes.

Two species of *Haplosplanchnus* were found in fishes seined from the immediate environment of infected snails. One occurred in a small parrot fish and the other, identified as *H. acutus,* was common in needle-fish and half-beaks. Further studies of these trematodes after the cercaria was found, indicated that of the two, *H. acutus* seemed most likely to be the adult of the larva in question. Not only was there closer agreement in the structure of the oral sucker, but the excretory patterns were identical, and most indicative of all, examination of the stomach contents of

the half-beak revealed still encysted metacercariae identical with those observed when the cercariae encysted in the laboratory. Metacercariae and excysted worms in the stomach with stages found in the intestine gave a complete series of development to maturity. It was also found that vegetation was a major portion of the diet of the half-beak, despite its habit of swimming close to the surface and evidently feeding there.

Description of Stages in the Life History

Sporocyst (Fig. 4)

Only the sporocyst generation producing cercariae was observed; it did not invade the digestive gland of the snail. The sporocysts were simple elongate and up to 0.825 mm. long and 0.14 mm. wide when measured alive without pressure. They were very motile, especially at what was evidently the anterior end where the body wall was thickened and contained yellowish gland cells. An indentation at that end apparently was a birth pore; a narrow canal could be seen leading from it into the body cavity of the sporocyst. A complex germinal mass, not shown in the figure, was seen at the posterior end. The excretory pores occur well toward the posterior end of the sporocyst. From each pore, a convoluted excretory tubule extends far into the anterior half of the sporocyst and barely turns posteriad before receiving an anterior and a posterior tubule which diverge and each terminates in a single flame cell. The excretory formula of the sporocyst is accordingly $2[(1)+(1)]$.

Cercaria (Figs. 1–3)

The cercaria develops in *Cerithium variabile* from which it was observed to emerge chiefly in the first two hours after sunrise. The larva is photopositive and swims almost continuously without regular rest periods. In swimming, it moves jerkily, tail first, and with the body contracted until almost spherical. The cercaria is biocellate and distomatous with a prominent rhabdocoele intestine extending well posterior to the ventral sucker. The tail bears on each side a row of eight finger-like processes and terminates with a somewhat different one. The body has a thick granular cuticle which lacks spines and contains much granular pigment scattered throughout both the parenchyma and the cuticle. Cephalic glands are lacking but the body posterior to the eye-spots is filled with dense cystogenous glands having cytoplasmic rodlets which appear first in the embryo as splinter-like structures. The oral sucker is non-protrusible but extremely mobile and with a conspicuous median lobe or papilla on the ventral lip. When expanded (Fig. 2a), the oral sucker is flattened, with ridges radiating outward from the mouth. There is a short prepharynx followed by a massive pharynx and a very muscular esophagus. The intestinal wall contains thick cells which stain pink with neutral red. The ventral sucker is situated near the mid-level of the body and is provided with six papillae around the opening which is scalloped.

The excretory vesicle is thin-walled, lacks concretions, and consists of a small ovoidal sac at the posterior end of the body with two long arms extending anteriorly to the pharyngeal level where each turns posteriorly as a ciliated recurrent tubule and receives an anterior and a posterior collecting tubule at the mid-level of the ventral sucker. Each collecting tubule is joined by three groups of flame cells,

two to each group, so that the excretory formula is $2[(2+2+2)+(2+2+2)]=24$ flame cells. There is a well developed sphincter at the posterior end of the vesicle and a distinct excretory chamber at the junction of the body and tail. In embryos (Fig. 3), the excretory pores are on the sides of the tail and well removed from its base.

Measurements in millimeters of heat-killed cercariae are as follows: body 0.178–0.243 long and 0.092–0.095 in maximum width which is somewhat anterior to the mid-level; tail 0.225–0.232 long exclusive of the terminal process and 0.028–0.030 in maximum width a short distance from the base; lateral processes of the tail are 0.038–0.054 long and the terminal one 0.052–0.055. The eye-spots are somewhat irregular in shape, the maximum dimension being 0.013–0.015. The oral sucker is 0.050–0.052 long and 0.054–0.060 wide, the ventral sucker 0.046–0.048 long and 0.049–0.052 wide. The prepharynx is 0.011–0.015 long and the pharynx 0.022–0.024 long by 0.027–0.030 in width.

Metacercaria (Fig. 5)

The cercaria may swim about for several hours before encysting and the detached tail swims vigorously for a considerable time thereafter. The cyst is spherical and from 0.103–0.108 mm. in diameter when measured in the fresh state under no pressure. The cyst wall is mooth, hyaline, evidently not laminated, and about 0.004 mm. thick. There is rapid dissociation of the eye-spots and their pigment becomes intermingled with that of the body.

The adult (Figs. 6 and 7)

Descriptions of the adult of *Haplosplanchnus acutus* have been given by Linton (1910, as *Deradena acuta*) and Manter (1937) and need not be repeated here. Manter's illustration is reproduced as Fig. 6 to bring illustrations of the various stages together in one plate. Observations on young adults from the half-beak, *Hyporhampus unifasciatus,* showed the excretory pattern to be precisely as described for the cercaria. Such specimens also yielded a clarification of the so-called neck glands described by Linton. Although Manter did not mention these glands in redescribing *H. acutus,* he stated that in *H. obtusus* they seemed to be yolk cells without yolk granules. From the study of living specimens, it is evident that the glands are much the same as the so-called salivary glands of other trematodes. They are in conspicuous masses in each half of the forebody; their ducts extend anteriorly in two bundles which converge and open (Fig. 7) on the characteristic papilla on the ventral lip of the oral sucker.

DISCUSSION

The first life cycle to be elucidated in a family of the digenetic trematodes sometimes reveals a close affinity with some other family, or that an established group is an unnatural one and may justify a concept of relationship so contrary to prevailing ones that many years must elapse and overwhelming evidence accumulate before the new concept becomes generally accepted. Evidently, no such information concerning the HAPLOSPLANCHNIDAE is provided by the present study. Both the cercaria and the adult differ in so many respects from those stages in other groups that not only does the validity of the family seem established but also that

there is no indication of a close relationship to other families. Although the cercaria resembles superficially the trichocercous larva of the LEPOCREADIIDAE, even casual examination reveals fundamental differences, especially in features of the excretory system. Morphology of the cercaria and adult and aspects of the life cycle suggest that the haplosplanchnids are as closely related to the fasciolids, echinostomes and psilostomes as to any other group. Poche (1926) derived the HAPLOSPLANCHNIDAE from the FASCIOLIDA and the aspidogastrids from the haplosplanchnids, presumably because of the form of the excretory vesicle, the rhabdocoele intestine, and the presence of a single testis. Resemblances in these respects could well be secondary and lead to the postulation of affinities that do not exist.

SUMMARY

Evidence is presented that the larva of *Haplosplanchnus acutus* is a biocellate, distome cercaria with a rhabdocoele intestine and a slender tail bearing eight pairs of lateral finger-like processes and a terminal one. The larva develops in simple sporocysts in the branchial region of *Cerithium variabile* and after emerging, it swims for a time and encysts in the open. Stages of development in the definitive host from still encysted metacercariae in the stomach to mature adults in the intestine were observed in natural infections of the half-beak, *Hyporhampus unifasciatus,* and adults were recovered also from needle-fish, *Strongylura* sp. The evidence suggests that a second intermediate host is lacking and that instead, metacercariae in the open are ingested with vegetation by definitive hosts which, for haplosplanchnids as a group, are fishes that are predominately herbivorous. There is no evidence of a close relationship of the HAPLOSPLANCHNIDAE to any other family.

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EXPLANATION OF PLATE

FIG. 1. Cercaria in ventral view with caudal appendages omitted from one side.

FIG. 2. Anterior end of living cercaria (a) expanded and (b) contracted.

FIG. 3. Sketch of a cercarial embryo showing definitive excretory tubes and pores.

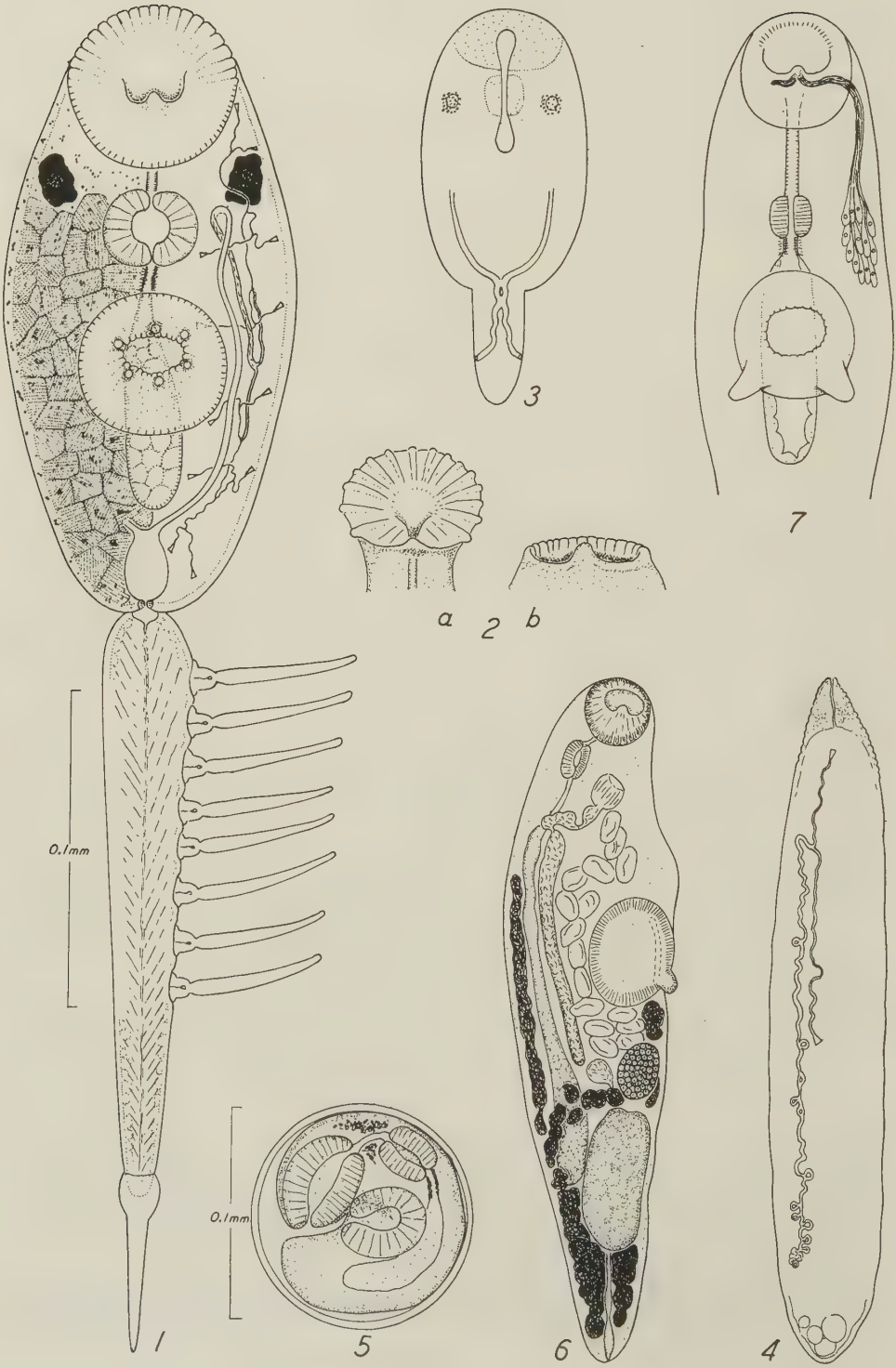
FIG. 4. Sporocyst with excretory system of one side.

FIG. 5. Metacercaria drawn from a living specimen.

FIG. 6. Adult of *Haplosplanchnus acutus* redrawn from Manter (1937).

FIG. 7. Sketch of a young adult of *H. acutus* showing the papillae of the suckers and the cervical glands as observed in a living specimen.

PLATE I



SEROLOGIC RESPONSE OF WHITE RATS TO *TOXOPLASMA* INFECTION[1]

Don E. Eyles

In an earlier paper from this laboratory, it was reported that about 20 per cent of the wild Norway rats of Memphis gave positive tests for toxoplasmosis using the Sabin-Feldman dye test. Only about three per cent of the same group of rats could be shown to harbor parasites which could be recovered after the inoculation of brain tissue into susceptible mice or guinea pigs (Eyles, 1952). In the discussion of that paper, it was pointed out that experimental infections in rats would have to be studied before the significance of the positive serologic reactions could be interpreted. The present paper reports serologic findings using the Sabin-Feldman test from albino Norway rats with experimentally induced *Toxoplasma* infections.

METHODS

The strain of *Toxoplasma* utilized in this study was recovered earlier from a wild Norway rat in Memphis (Eyles, 1952), and was maintained by serial intraperitoneal transfer of peritoneal exudate in white mice. Young adult albino Norway rats, reared in the National Institutes of Health animal colony, were used for the experiments.

The experimental procedure was as follows:

1. Selected rats were bled from the heart under ether anaesthesia, using usually a trace of heparin, and the plasma was separated for pre-infection serologic study. Two susceptible white mice were each inoculated intraperitoneally with 0.1 cc. of blood from the rat.

2. The rats were then inoculated intraperitoneally with *Toxoplasma* organisms secured by diluting the peritoneal exudate from infected mice with physiologic saline. The number of organisms was counted with a hemocytometer and 5,000 or 50,000 organisms were injected.

3. Blood was taken from the heart at two to four day intervals for the first 12 days of the infection (in two instances there was an eight-day interval). Part of the blood (0.1 cc.) was injected intraperitoneally into each of two susceptible mice in an effort to demonstrate a parasitemia. The remainder of the blood was centrifuged and the plasma preserved for serologic testing.

4. Subsequently the rats were bled weekly for two months, every two weeks for two months, then monthly until a year had elapsed. The rats remaining at this time were then bled every three months. About 0.5 cc. of blood was taken and all of the plasma kept for serologic testing. There were some deviations from this bleeding schedule due to the fact that some rats were difficult to bleed and became increasingly so due to scar tissue formation from repeated heart puncture.

5. At the end of six months five of the rats were sacrificed. After autopsy examination, brain tissue suspension was injected into white mice to see if *Toxoplasma*

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infection would follow. The white mice which survived one month were sacrificed and tissues subinoculated to a second set of clean mice which were sacrificed and autopsied at the end of another month.

6. The remaining rats were followed until they died, in order to determine how long the serologic findings remained positive.

The serologic procedure used in this investigation was the dye test of Sabin and Feldman (1948) applied essentially as described in their paper. Due to the small volume of serum available, tests on undiluted serum had to be omitted in a few tests. All tests prior to infection and in control rats were performed on undiluted plasma. Four-step dilutions were employed. Controls of a known positive serum, diluted beyond the end point were used in each series of tests and tests were repeated if the controls were markedly discrepant.

OBSERVATIONS

Parasitological demonstration of the infection in inoculated rats. Rats inoculated with *Toxoplasma* showed no visible signs of illness and none died from the in-

TABLE 1.—*Demonstration of parasitemia in experimentally induced* Toxoplasma *infections in albino Norway rats by inoculation of blood into susceptible mice at intervals after inoculation*

Rat number	Size of inoculum (Number of organisms)	Result of subinoculation of 0.1 cc. of blood into mice related to days after inoculation											
		0*	1	2	3	4	5	6	7	8	9	10	11	12
A	5,000	–				+								+
B	5,000	–			–		–							
C	5,000	–				–				–		–		
D	5,000	–				+			+		–			
E	5,000	–				–			–		+			
F	5,000	–				–			–		+			
G	5,000	–				–			+		+			
H	5,000	–				+			+		+			
I	5,000	–				–			+		–			
J	50,000	–				+								+
K	50,000	–			+		–							
L	50,000	–				+			–		+			
M	50,000	–				+			+		–			
N	50,000	–				–			–		–			
O	50,000	–				–			–		–			
P	50,000	–				+			–		+			
Q	50,000	–				–			+		–			

* Before inoculation.

fection. In order to demonstrate that infection had occurred, 0.1 cc. samples of blood from the inoculated rats, taken at varying times after inoculation, were injected intraperitoneally into each of two young white mice. Our experience, and the experience of other workers, has been that young laboratory mice are rarely if ever spontaneously infected with *Toxoplasma,* the only exception to this experience being that of Mooser (1950). If these mice became infected it indicated that a parasitemia had developed in the rats and this was considered evidence that the rats had contracted the infection.

Infection developed in some of the mice inoculated from all but 4 of the 17 white rats as shown in Table 1. In 7 of 15 instances, parasitemia was demonstrated on the fourth day after inoculation; in 6 of 12 instances parasitemia was demonstrated on the seventh day after inoculation or the ninth day after inoculation. Parasitemia was demonstrated on the third day in one case and on the twelfth day in two cases. In summary, it would appear that between the third and 12th day parasitemias were

demonstrable about half of the time. Parasitemias might have been detected after the 12th day if subinoculations had been made. Similar irregularly demonstrable parasitemias were described in rats by Jacobs and Jones (1950) and by Ruchman and Fowler (1951).

The fact that mice inoculated from four of the rats did not develop parasitemias cannot be taken to indicate that these rats had not become infected, for the production of infection in the mice was irregular in the other cases. Frequently, one of the two mice simultaneously subinoculated would succumb to *Toxoplasma* infection. Finally, no marked difference as regards parasitemia was evident when the groups inoculated with 5,000 and 50,000 *Toxoplasma* organisms were compared.

Serologic tests prior to inoculation: Plasma from the 17 rats taken prior to the inoculation all produced negative dye test reactions. In an effort to determine if this was always true of young adult rats secured from the same source, 33 additional clean rats were bled and dye tests conducted. These also were all negative with the exception of one rat which produced a positive reaction with undiluted plasma.

From the above evidence, it would appear that the laboratory rats did not often possess natural antitoxoplasmic factors in their blood. It would also appear that spontaneous infection had not occurred frequently in our group of rats. The results to be reported below show that in all instances the inoculation of *Toxoplasma* produced a serologic response.

Development of serologic response: Figure 1 graphs the response of 17 rats to *Toxoplasma* infection as measured by the Sabin-Feldman dye test using the homologous organism. Analysis of these data disclosed the following:

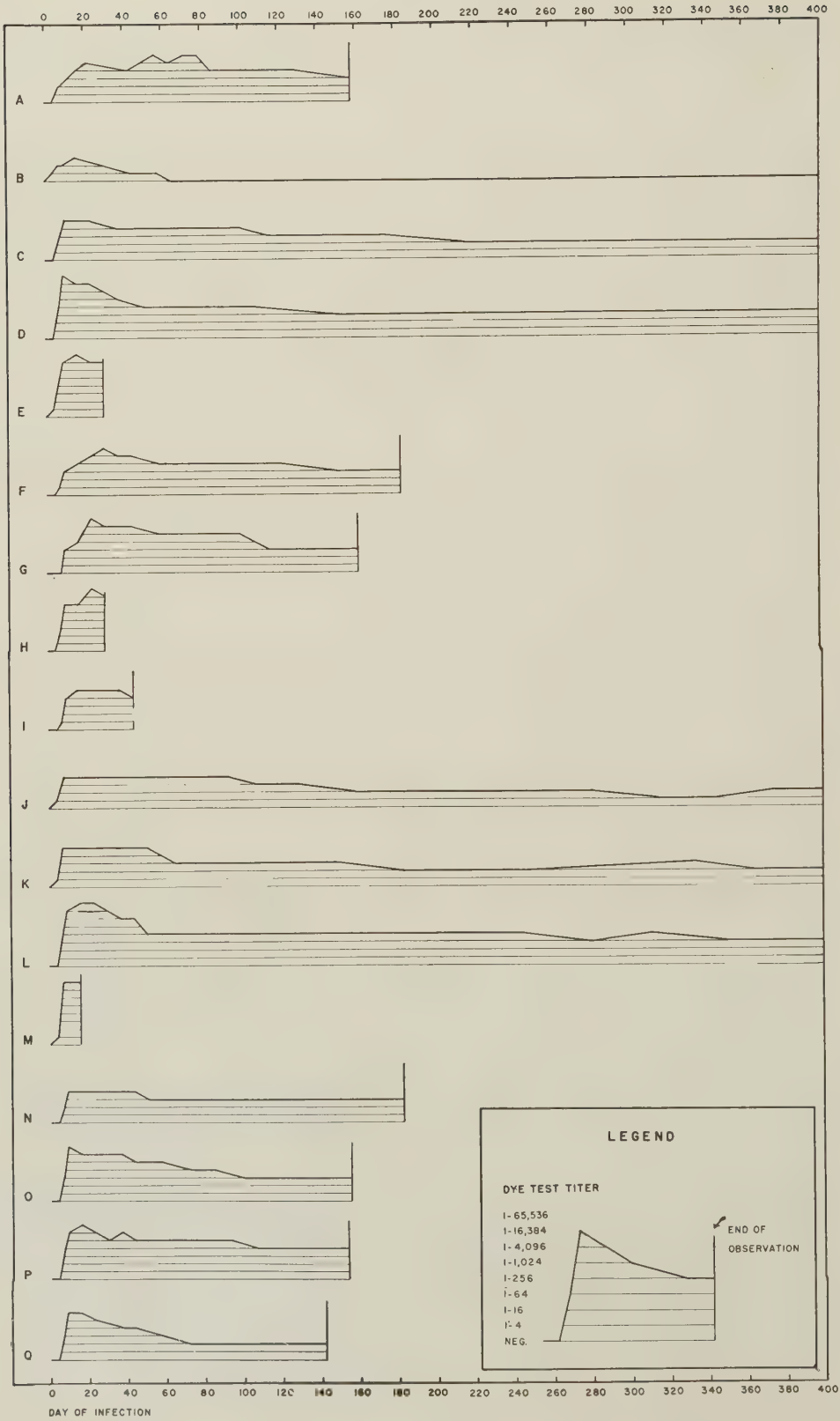
1. All rats inoculated showed a serologic response. This response was immediate in that 29 per cent showed positive titers as early as the fourth post-inoculation day and the remainder were positive by the seventh day.

2. Peak titers were reached between the 7th and the 30th day. Most frequently the peak titer was reached between the 9th and the 16th day, the median day of peak titer being the 15th. In some instances the peak titer was of short duration; whereas, in others it persisted for several days or longer. It must be pointed out that, since tests were not run daily, we may not have measured the true peak in all cases.

3. The peak titer varied from a dilution of 1 : 64 to 1 : 65, 536. Most frequently the peak titer approached the latter dilution and the median peak titer was 1 : 4096.

4. Titers fell more or less rapidly soon after the peak was reached. At two months the median titer was 1 : 256, or one-eighth the peak titer. After five to six months the median titer was 1 : 64, then after one year, 1 : 16. In other words the fall in titer by the end of two months was the approximate equivalent of the fall during the subsequent 10 months.

5. With one exception, rats continued to produce positive dye tests as long as they lived. Five rats were followed more than the 400 days shown on the chart. Rat C was followed until the 476th day at which time the titer was measured as 1 : 4. Rat D was followed for 620 days at which time the titer was 1 : 256. Rat J was observed for 414 days and the last reading was 1 : 16. Rat K was followed 543 days at which time it showed a titer of 1 : 16. Rat L was followed 620 days and the titer at the end of this period was 1 : 64.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A
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1-65,536
1-16,384
1-4,096
1-1,024
1-256
1-64
1-16
1-4
NEG.
END OF OBSERVATION
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400
DAY OF INFECTION

6. The exception to the above was rat B. This rat was inoculated with 5,000 *Toxoplasma* and no parasitemia was demonstrated. Its peak titer was 1:64 and the titer rapidly fell until it was negative on the 65th day. Subsequently, rat B was followed until the 634th day and remained serologically negative throughout this period.

7. Figure 1 shows several instances in which titers fell slightly then subsequently rose. We cannot be sure that this is not due to technical factors, as it was usually impossible to repeat the tests more than once or twice due to the limited amount of plasma.

8. Judging from the serologic response, the rats in which parasitemia was not demonstrated, did acquire *Toxoplasma* infections. Rat B which responded only briefly and never reached a high titer may be an exception, but even here the duration of the positive reaction (64 days) would seem to indicate more than a response to dead antigen.

9. There were no obvious differences in serologic response between the rats receiving the inoculum of 5,000 organisms and the rats receiving 50,000.

Results of subinoculation of brain tissue from rats sacrificed after six months: At the end of six months rats F, N, O, P, and Q were sacrificed. Impression smears from the brain, liver, spleen, lungs, and viscera were examined from each rat and no parasites were seen. The brains of the rats were ground in about 10 cc. of physiological saline solution and one cc. of the mixture injected intraperitoneally into each of three clean mice. At the end of four weeks the surviving mice were sacrificed and tissue suspensions of brain and liver injected intraperitoneally into three additional clean mice.

As a result of the above procedure, two rats were shown to be infected. In one instance mice of the first passage showed the infection and in the second, mice of the second passage died of toxoplasmosis. It cannot be concluded that the above proved the absence of organisms in the rats which gave negative results. In the first place only a portion of the brain was put into the mice and some organisms may not have survived the suspending procedure. This process, however, is the equivalent of the method we used in a previous study (Eyles, 1952) in which we isolated *Toxoplasma* from wild rats.

DISCUSSION

The experiments reported were designed to provide data for better understanding of the findings from studies on wild Norway rats. The experimental studies demonstrate that few young rats possess either natural antitoxoplasmic factors or have experienced *Toxoplasma* infection. The studies further demonstrate that infection with *Toxoplasma* produced an immediate serologic response and that this response in most instances persists for a long time. From this one might suppose that the positive serologic findings of our study on wild rats (Eyles, 1952) all represent *Toxoplasma* infection. This hypothesis should be tested by studying the reaction of wild Norway rats with and without positive serologic titers to experimentally induced *Toxoplasma* infections.

FIG. 1. Development of dye test response in albino Norway rats with experimental infections of *Toxoplasma*. Rats A–I received inoculum of 5,000 organisms and remainder, 50,000 organisms. Day 0 represents day of inoculation.

The life span of laboratory rats is approximately three years (Farris, 1949). Most likely the life span in nature of the Norway rat is much shorter due to the natural factors which limit length of life. If this be so, it is likely that most of the wild rats found serologically negative had never experienced *Toxoplasma* infection, for in the experimental studies five out of six rats followed for 400 to 600 days remained positive throughout the period of observation.

In view of our experience in the present study in which rats sacrificed at six months produced infections in mice in only two of five cases, it is not surprising that we failed to isolate organisms from many rats with positive serologic titers in our wild rat study (Eyles, 1952). On the other hand Ruchman and Fowler (1951) were able to recover *Toxoplasma* from the brains of all rats sacrificed up to two years after inoculation. A possible cause for this apparent discrepancy in findings is that the inocula used by Ruchman and Fowler were undoubtedly much larger than in the present study, since they injected one cc. of a 10 per cent infected brain suspension as compared with our 5,000 or 50,000 organisms.

The present study fails to enlighten us as to a possible explanation of the isolation of *Toxoplasma* from wild rats that were serologically negative. It is conceivable that the isolations were made from rats in the early stages of infection before serologic response had occurred; however, the immediate response of the experimental rats would make this appear unlikely. Our experience was similar to that of Jacobs *et al.* (1952) who isolated *Toxoplasma* from wild pigeons which were serologically negative.

That some rats may become serologically negative after exposure to infection is illustrated by our Rat B, but in this case we were not able to demonstrate parasitemia. Unfortunately no post mortem studies on this rat were possible due to the fact that it was deteriorated when found dead 634 days after inoculation.

SUMMARY

Seventeen young adult albino Norway rats were experimentally infected by the intraperitoneal injection of 5,000 or 50,000 organisms from a strain of *Toxoplasma* originally isolated from a wild Norway rat. Thirteen showed parasitemia which was taken to indicate that infection had occurred. None died of *Toxoplasma* infection or showed signs of illness.

All of the rats gave negative responses to the Sabin-Feldman dye test prior to inoculation. All responded positively after inoculation. Response occurred by the fourth to seventh day of the infection. The peak titer first occurred on about the 15th day (median). Median peak titer was 1:4000. Median titer after two months was 1:256 and after one year 1:16. Five rats followed over 400 days all remained positive until death, the maximum followup being 620 days. In only one rat was serologic response different. In this rat the peak titer reached was 1:64 and the serologic response was absent by the 65th day after inoculation.

Brain suspensions from five rats sacrificed at six months were injected into clean white mice (two passages). By this process organisms were demonstrated to exist in two of the five rats.

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PARASITISM OF THE GRASSHOPPER, *CHORTOPHAGA VIRIDIFASCIATA* (DEGEER) (ORTHOPTERA: LOCUSTIDAE), BY DIPTEROUS LARVAE[1]

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Field collections of nymph and adult *Chortophaga viridifasciata* (DeGeer) from Knox and Anderson counties in Tennessee have shown a rather heavy incidence of parasitism, particularly in nymphs collected in late fall and winter. Two primary parasites have been found, namely: *Ceracia dentata* (Coq.) (Diptera: Tachinidae), and *Sarcophaga hunteri* Hough (Diptera: Sarcophagidae). In addition, the puparia of *C. dentata* have yielded a hyperparasite, *Brachymeria tegularis* (Cress.) (Hymenoptera: Chalcididae).

The literature has revealed only two definite reports of *C. dentata* as a parasite of *Chortophaga.* One of these is listed by Coquillet (1897) from St. Louis, Missouri; the second is reported by Treherne and Buckell (1924) from Ontario and British Columbia. *S. hunteri* was reared from *Chortophaga* by Kelly (1914), and *B. tegularis* from *C. dentata* (= *Acemyia dentata*) by Burks (1940).

We have been primarily concerned with the life history and development of *C. dentata* which is by far the most abundant parasite. Infestation first became evident with the appearance of puparia in cages where grasshoppers are maintained for laboratory use. Three distinct larval stages have been recovered from dissections of nymphal and adult grasshoppers, and a fourth larval stage (free) has been found.

Stage I.

The buccopharyngeal armature in this stage (figs. 1, 2, 3) consists of a single median mouth-hook with a rather well developed pair of dorsal wings and a small ventral projection. There is a well developed buccopharyngeal plate (fig. 3) imbedded in the integument ventral to the mouth-hook.

Stage II.

In the stage II larva, a ventral wing has formed, a pair of mouth-hooks is present, and the buccopharyngeal plate of the stage I larva has been lost. The mouth-hooks of stage II larvae (figs. 4, 5) show more sclerotization than do those of

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stage I. The last abdominal segment bears the posterior spiracles, which do not project from the body of the larva. Their loci are indicated by an arrow in figure 6. The spiracular structure of this larva does not differ greatly from that of the previous stage and figures 6 and 7 serve to illustrate both stages.

Stage III.

The buccopharyngeal armature of this stage (figs. 8, 9) is more heavily sclerotized than in previous stages and a pair of cuticular sensorial organs are easily visible on the apex of the larval head. The spiracles (fig. 10) show a marked difference from those of the earlier stages. They have increased greatly in size and project from their segment on well sclerotized bases. A well defined spiracular button is evident.

Stage IV.

This is the larva which emerges from the host and pupates. It is morphologically indistinguishable from stage III.

Puparium.

The puparium is dull reddish-brown in color and varies considerably in size. Maximum and minimum dimensions are indicated on the side view of the puparium shown in figure 11. A head-on view (fig. 12) shows a vertical groove and a pit, representing the locus of the larval armature, and a horizontal fracture line where the puparium may split at the end of pupation. A ventral view of the posterior end (fig. 13) shows two heavily sclerotized spiracular projections and, at the base of the spiracles, the anal scar.

Biology.

Tachinids are known to infest their hosts in a number of ways (Sweetman, 1936), but the method employed by *Ceracia* has not been determined. The earliest larvae which we have recovered (stage I) are free in the hemocoel and usually lie close to one of the larger air sacs. A number of these may occur in each host (see below); we have found as many as six in a single nymph. In a number of instances larvae which had apparently died in the process of molting were found.

According to Snodgrass (1935), the mouth-hooks of cyclorrhaphous larvae are not renewed after the third molt. The armature is renewed in our material between stages I and II, stages II and III and stages III and IV. Stage III larvae molt and the resulting larvae (stage IV) emerge from the hosts. The implication is that all larval stages are represented. It is felt, however, that more work must be done before this can be stated with certainty. With adult flies at hand, we have attempted to infest grasshoppers under laboratory conditions, but we have been unable to do so.

Most of the exuvia which result from the molting of stage I and II larvae are entangled with the Malpighian tubules of the host. We have on a number of occasions, however, found exuvia free in the hemocoel. The exuvia are transparent and might easily be overlooked were it not for the glistening black color of the buccopharyngeal armature.

Stage II larvae are loosely attached by the spiracular plates to one of the larger tracheal trunks or to an air sac. The larval skin which is shed by this larva is

easily distinguished from that shed by stage I larvae since a buccopharyngeal plate is conspicuous in one of these and absent in the other.

Stage III larvae are firmly attached to the host, the most usual points of attachment being the thoracic or first abdominal spiracles (fig. 14). When this larva molts, the exuvium remains attached and the resulting larva leaves the host. Newly emerged larvae have been observed but the act of emergence has not been witnessed. The emerged larva (stage IV) is negatively phototrophic and remains motile for a variable period of time before pupation. Pupation time is about two weeks at room temperature.

As is noted above and in figure 11, puparia show a wide variation in size. In addition, the positioning of the spiracles ranges from near horizontal as shown in figure 11 to angled at nearly 45° dorsally. Adults from the smaller puparia are, of course, smaller than are those from the larger ones, and series of sizes have been obtained. Coquillett (1897) explains this size variation as follows: The number of tachinid larvae introduced into the host is often close to or even greater than that which the host can support. "Owing to this fact, a great many tachinid larvae must necessarily perish for want of food, while several which have barely had a sufficient quantity to keep them alive will be considerably dwarfed in size, as compared to their more fortunate brothers, and thus it will sometimes happen that some of the adult flies will be only one-half, and in rare instances, even only one-third as long as others which were the progeny of the same parent."

We are indebted to Drs. Curtis Sabrosky and B. D. Burks of the U. S. National Museum for the identifications of reared adult parasites.

SUMMARY

1. A new locality is reported for two dipterous parasites, *Ceracia dentata* (Coq.) (Tachinidae) and *Sarcophaga hunteri* Hough (Sarcophagidae), of the grasshopper, *Chortophaga viridifasciata* (DeGeer). In addition, the presence of a hymenopteron hyperparasite, *Brachymeria tegularis* (Cress.) (Chalcididae), is noted from *C. dentata*.

2. Four larval stages and the puparium of *Ceracia dentata* (Coq.) are described. Observations on the life history of this species are included.

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PLATE I

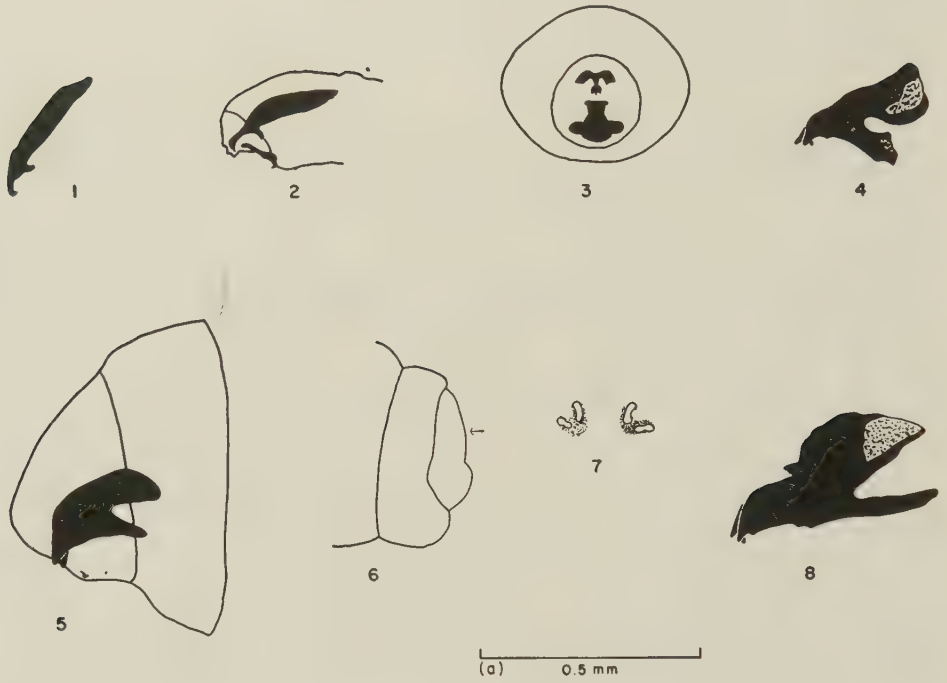
1
2
3
4
5
6
7
8
(a) 0.5 mm
9
10
12
(b) 1.0 mm
Max. 6.75 mm
Min. 3.25 mm
Max. 3.00 mm
Min. 1.00 mm
11
13

PLATE Ia

PLATE I

Ceracia dentata (Coq.). Scale (a), figs. 1 through 10; scale (b), figs. 12 and 13.

FIG. 1. Lateral view, buccopharyngeal armature, stage I larva.
FIG. 2. Lateral view, buccapharyngeal armature *in situ,* stage I.
FIG. 3. Frontal view, buccopharyngeal armature *in situ,* stage I.
FIG. 4. Buccopharyngeal armature, stage II.
FIG. 5. Buccopharyngeal armature *in situ,* stage II.
FIG. 6. Lateral view, posterior end, stage II. Arrow indicates loci of posterior spiracles.
FIG. 7. Posterior spiracles, stage II, felt chambers are stippled.
FIG. 8. Lateral view, buccopharyngeal armature, stage III.
FIG. 9. Frontal view, buccopharyngeal armature *in situ,* stage III, showing sensorial organs.
FIG. 10. Posterior spiracles, stage III.
FIG. 11. Puparium, side view.
FIG. 12. Puparium, frontal view.
FIG. 13. Puparium, ventral view of posterior end.
FIG. 14. Dissection of a *Chortophaga* nymph showing two stage III larvae attached at the mesothoracic spiracles. Fixation: Carnoy's (with chloroform).

THE SIZE ATTAINED BY *HYMENOLEPIS DIMINUTA* IN DIFFERENT HOST SPECIES

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It might be surmised that the size attained by a tapeworm in a particular vertebrate host will be related to the size and species of the host animals. However, little quantitative data to support this view are actually available. Larsh (1946) showed that *Hymenolepis nana* attained a greater length in the hamster than in the mouse; in these experiments absolute comparisons are still open to the criticism that the two groups of hosts harbored quite different numbers of worms. Beck (1951) has demonstrated the usefulness of singly-established worms in the study of the biological relationships of *Hymenolepis diminuta.* In the present experiments individual hosts of several species were infected with single cysticercoids of *H. diminuta,* and an evaluation was made of the size attained in these various hosts. Volume of tapeworm tissue was used as the unit of measure of size attained.

MATERIALS AND METHODS

The tapeworm eggs for all experiments were obtained from the droppings of one rat infected with *H. diminuta* of the Rice Institute strain. Cysticercoids were obtained by feeding eggs to laboratory-reared *Tenebrio molitor.* In each series of host animals of the same species the cysticercoids used for infection were obtained from one beetle. Each host was lightly anesthetized with ether and a single cysticercoid was administered per ora with a pipette. All hosts were autopsied twenty days after infection. (The senior author has found the pre-patent period of *H. diminuta* to be 19 to 21 days in the hosts used in the present work.) The worms were washed in several changes of Tyrode's solution and blotted carefully on filter paper. Volume of worm tissue was determined by adding each worm to a 5.00 ml. burette, graduated to 0.01 ml., partially filled with Tyrode's solution. The difference in readings before and after addition of each worm was recorded as tissue volume. Tyrode's solution was used because it has been previously shown that the wet weight of *H. diminuta* does not change in this medium (Chandler, Read, and Nicholas, 1950). Duplicate determinations with the individual worms of one series of ten worms yielded a maximum error of 0.04 ml. No other measurements of worm size were made.

EXPERIMENTS

In a preliminary experiment twelve gray mice, *Mus musculus,* were given single cysticercoids. At autopsy twenty days later only one host was found to be harboring a worm. In a second experiment thirty-six albino mice, 60 to 73 days old, were used. Half of this group were males and half females. Six males and six females were given cysticercoids. The remaining twenty-four mice were given 0.05 ml. of 10% tincture of opium intraperitoneally; thirty minutes later each of these mice was given a cysticercoid. At autopsy the mice not treated with opium were free of worms while every individual in the treated group harbored a single

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H. diminuta. Hamsters and albino and hooded rats were readily infected without other treatment. These hosts were 95–105, 130–148, and 140–175 days old, respectively. The data obtained from opium-treated mice, hamsters, and the two strains of rats are summarized in Table 1.

TABLE 1.—*The size attained in twenty days by singly established* Hymenolepis diminuta *in various hosts. Host weights and worm volumes are expressed in grams and cubic milliliters, respectively. S. E. = Standard error.*

(Female) Albino Mice			Albino Mice (Male)			Hamsters (Male)			Albino Rats (Sprague-Dawley) (Male)			Hooded Rats (Long-Evans) (Male)		
Host No.	Host Wt.	Worm Vol.	Host No.	Host Wt.	Worm Vol.	Host No.	Host Wt.	Worm Vol.	Host No.	Host Wt.	Worm Vol.	Host No.	Host Wt.	Worm Vol.
1	32.0	0.26	13	27.0	0.27	1	109	0.44	1	325	1.30	1	430	1.25
2	30.0	0.27	14	30.0	0.29	4	144	0.54	2	401	0.95	2	458	1.16
3	28.0	0.31	15	29.0	0.31	7	109	0.56	3	352	1.21	4	481	1.37
4	27.5	0.25	16	28.5	0.29	9	133	0.80	4	358	1.11	6	429	1.24
5	30.0	0.24	17	26.0	0.34	10	123	0.58	4a	347	1.25	7	444	1.06
6	25.5	0.30	18	24.5	0.29	11	118	0.49	5	391	0.89	8	439	1.41
7	26.5	0.28	19	31.0	0.36	12	130	0.60	6	407	0.99	10	469	1.38
8	29.0	0.26	20	25.0	0.27	13	122	0.67	7	382	1.18	11	503	1.52
9	30.0	0.24	21	24.0	0.28	16	125	0.48	7a	432	0.88	12	402	1.69
10	27.0	0.22	22	25.5	0.31				8	398	0.72	15	461	1.23
11	25.0	0.26	23	29.0	0.30				9	300	1.27			
12	24.0	0.20	24	31.0	0.29				9a	366	1.28			
									12	436	1.50			
Av. = 27.8		0.26		27.5	0.30		123	0.57		377	1.11		452	1.33
S.E. = —		0.023		—	0.020		—	0.071		—	0.180		—	0.142

DISCUSSION

The failure of *H. diminuta* to establish itself in mice unless the hosts are treated with opium may be interpreted in terms of Larsh's (1947) studies on the relation of intestinal emptying time to the number of *H. nana* established in these hosts. It seems evident that the effect of intestinal emptying time on the establishment of *H. diminuta* is even more marked than with *H. nana.* This may explain the fact that *H. diminuta* only rarely occurs in house mice and may be regarded as a probable explanation of apparent host specificity in this particular case. These findings indicate the ambiguity of the simple term "host-specificity" and emphasize the fact that there are probably few generalizations which will apply in explaining physiological parasite-host specificity in terms of mechanism without a detailed study of the particular host and parasite under consideration.

There is no evidence of significant difference in size attained by worms from male and female mice. It is obvious that there are significant differences in the size attained by *H. diminuta* in different host species. This is related in a general way to the weight of the host. More specifically, it is probably related to the size of the small intestine and perhaps to other physical features of the intra-intestinal environment.

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FURTHER STUDY ON *LYMNAEA STAGNALIS* (L.) AS A SNAIL HOST FOR *FASCIOLOIDES MAGNA* (BASSI, 1875) (TREMATODA)

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In our previous note (1953) we had reported the recovery of the sporocyst and young rediae from the snail *Lymnaea stagnalis*. The intramolluscan stages were subsequently followed in the same snails and completed experimentally in the laboratory.

MATERIAL AND METHOD

The eggs and the specimens of *Fascioloides magna* were obtained from the liver of an elk (*Cervus canadensis*) from Burwash, Ontario, in September 1952. Eggs were dissected from both the uteri of the flukes and from the cysts in the liver. They were incubated with spring water at room temperature, 70° to 75° F.

Young laboratory bred *Lymnaea stagnalis,* from five to ten weeks old, were used for experimental infections and the snails were subsequently kept at the same temperature as the eggs.

Rediae were fixed with Gilson fixative and stained with alum cochineal.

EGGS AND MIRACIDIUM

Eggs obtained from the cysts in the liver were kept in Petri dishes (140 mm. in diameter and 40 mm. in height) with plenty of water added. No subsequent changes of water were made, except fresh water was added twice during the whole course of development. The percentage of eggs undergoing development was extremely high. Practically every egg contained a miracidium. Well developed miracidia and a few hatchings were seen at about six weeks. Hatching of the eggs continued for many weeks. At the 85th day, there were still many eggs with miracidia in the culture. They were removed to syracuse dishes with fresh water added. Because of the stimulation from the fresh water, hatching began in about half an hour and continued during the following three hours of observation.

Eggs from the cysts as well as those dissected from the uteri were also incubated under different conditions: in Petri dishes, in test tubes and with varying frequency of water changing. Eggs developed more slowly in test tubes and with water less frequently changed. In cultures with water changing every two days, eggs with well developed miracidia were seen in 19 to 26 days. This might be related to the oxygen supply as suggested by Swales (1935).

DEVELOPMENT IN SNAIL HOSTS

Twenty-seven snails, about five weeks old, were each exposed individually to from four to ten miracidia. Twenty snails were available for examinations and four were found infected.

One sporocyst and two rediae were found in a snail 35 days after exposure. A single redia was found in the vicinity of the respiratory chamber of a snail two months and 17 days after infection. Another single redia was found in another snail three months and 12 days after infection. The intramolluscan stages were com-

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pleted in a snail five months and four days after exposure. The growth of that snail was very slow and it showed a sign of dying. It was dissected and cercariae were found emerging from the opening of the respiratory chamber. Many rediae were found in the respiratory chamber and the liver. Some of the cercariae succeeded in encystment on the bottom of a dish. No cysts were found on the wall of the glass container in which the snails were raised. It appeared that the cercariae were just beginning to leave the host. The number of cercariae found in the rediae varied from one to six. The one with six cercariae measured 1.102 mm. by 0.224 mm.

Another lot of 31 snails about 10 weeks old were exposed to mass infection with miracidia. Eleven of them were found infected. The details of our findings are summarized in Table 1.

TABLE 1.—*Results of the examination of* L. stagnalis, *about 10 weeks old, exposed to mass infection of* F. magna *miracidia*

Time after infection	Number of snails examined	Number of snails infected	Results
2 months and 24 days	5	None	
3 months and 5 days	2	1 infected (No. 1)	6 young rediae found.
4 months and 8 days	2	1 infected (No. 2)	4 rediae found.
4 months and 11 days	2	1 infected (No. 3)	1 redia found.
4 months and 17 days	6	1 infected (No. 4)	2 rediae found.
4 months and 20 days	1 died	Infected (No.5)	Numerous rediae found: mostly in mantle, in wall of respiratory chamber, some along digestive tract, some in liver, some in margin of foot and also embedded in all the soft tissues.
4 months and 23 days	1	Infected (No. 6)	Numerous rediae found: in mantle, attaching to digestive tract and other organs, some in soft tissue just dorsal to foot, and a small number in liver.
5 months	2	1 infected (No. 7)	1 medium and 2 small rediae found in mantle.
5 months and 6 days	2	1 infected (No. 8)	About a dozen medium-sized rediae found in mantle, some with germinal masses.
5 months and 10 days	1 died	Infected (No. 9)	A few dozens of large and small rediae found in mantle, small germinal masses seen.
5 months and 23 days	1 died	Negative	
5 months and 26 days	1 dying	Infected (No. 10)	Numerous large and small rediae found in mantle, margin of foot and a few in liver; in some rediae small germinal masses found.
6 months	1 died	Negative	
6 months and 3 days	2	Negative	
6 months and 6 days	1 dying	Infected (No. 11)	Many large and small rediae found attaching to wall of respiratory chamber and in the mantle.
6 months and 12 days	1	Negative	

Rediae recovered from infected snails No. 1 to 4 were probably mother rediae. There were only a few rediae recovered from each snail and no embryos were found in those rediae.

Rediae recovered from snail No. 5 were in various sizes. Some of them were fairly large. They were not in good condition when fixed. However, some of them were stained and mounted and many were found with developing embryos. A few very young cercariae were present.

About two-thirds of the rediae from snail No. 6 were stained and mounted. Many of them were with embryos in various stages of development. Since the

daughter and mother rediae are very similar, their stages are difficult to determine. However, there were five specimens with well developed embryos which confirmed their stages: two mother rediae, one with two daughter rediae and one with one daughter redia; three daughter rediae, one with three cercariae and the other two each with one cercaria.

Some rediae from snail No. 11 contained developing embryos. Four of them were with well developed daughter rediae. The number varied from one to three.

DISCUSSION

Miracidia attacked *Lymnaea stagnalis* readily. But not all of them succeeded in penetrating the snail. Some of them were seen hanging to the snail but eventually became exhausted; dropped off and died.

The development of *F. magna* in *L. stagnalis* was much slower than in *Fossaria parva* and *Stagnicola palustris nuttalliana,* Swales (1935), at about 75° F.; *Fossaria modicella rustica* and *Pseudosuccinea collumella,* Krull (1933a), at 80° to 85° F.; *Galba bulimoides techella,* Sinitsin (1930) and Krull (1933b); and *Fosaria modicella,* Krull (1933b). The size of the daughter redia is much smaller than that developed in *F. parva,* only about the size of those in *S. palustris nuttalliana.*

Rediae developed in the respiratory chamber, liver and mantle. Often numbers of rediae were found in channels (possibly lymphatic) running through the tissues parallel to the peripheral margin of the foot. The development inside the mantle and foot appeared to be slower. The liver of the snail was less frequently infected. In cases where livers were infected, the number of rediae found was not so great as compared with the number found in other organs.

The development of the mother rediae was slow. The number of rediae found in the infected snails was never great even up to about four months after infections. This fact might suggest that the parasites adapt themselves to overwinter in the intermediate host as early larval stages when the miracidia enter the snail host in the fall. However, more work should be done on this ecological phenomenon.

Heavy infections appeared to be responsible for the death of the infected snails. Therefore, *L. stagnalis* might not be a normal intermediate host.

SUMMARY

1. The eggs of *F. magna* from the liver of an elk incubated at room temperature (70° to 75° F.) began to hatch in six weeks.

2. Practically all the eggs obtained from the cyst cavities in the liver of an elk developed into miracidia, whereas many of the eggs from the uteri of the parasites did not develop.

3. The development of the mother rediae was very slow, taking over four months for the completion.

4. The intramolluscan stages of *F. magna* have been completed in laboratory bred *Lymnaea stagnalis* in five months and four days. The number of cercariae in each daughter redia varied from one to six.

5. Rediae developed in the respiratory chamber; in the tissue of the liver, mantle and margin of the foot. Rediae were also seen attached loosely to the external surface of the digestive tract and other soft tissues.

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A REDESCRIPTION OF THE AMERICAN LIZARD MITE, *GECKOBIELLA TEXANA* (BANKS), 1904, WITH NOTES ON SYSTEMATICS OF THE SPECIES (ACARINA: PTERYGOSOMIDAE)

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INTRODUCTION

The American lizard mite, *Geckobiella texana,* was first described September 24, 1904, by Nathan Banks, who placed the species in the genus *Geckobia* Megnin, 1878, which he misspelled as *"Gekobia."* Banks noted that this mite was collected from the lizard *Sceloporus floridanus* in this country and presented six sketches including a ventral view of a slightly engorged female, a dorsal and ventral view of the "beak," and detailed drawings of the palpi, claw, and chelicera. These sketches were quite small and aid only slightly in identifying the species.

Banks (1906) redescribed *Gekobia* (= *Geckobia*) *texana* and indicated that he was describing a new species. This publication included a more detailed description and cited Austin, (Travis County) Texas, as the place of collection. Following the locality data was the word "May." It is not clear whether this identified the month of collection or the name of the collector. This redescription has created some confusion as to the exact year that should be associated with the first description of this species. It is assumed that Banks did not purposely change the spelling of the generic name but that a *lapsus calami* persisted in his writings through subsequent papers in 1906, 1907, and 1915. Examination of the journals in which the two descriptions were published leaves no doubt as to the exact date of publication. Although the date 1905 appeared on the title page of volume 28 of the Proceedings of the United States National Museum, the exact date of the appearance of the first paper under discussion was September 24, 1904, as stated on page one of the table of contents of volume 28. Another cause for confusion is that Banks indicated in his 1906 paper that he was describing *G. texana* for the first time. The volume of the Proceedings of the Entomological Society of Washington in which this article appeared is dated 1905, but the exact date of the release was January 10, 1906, as stated on page 152. Thus it is apparent that the date which should be associated

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with the original description of the lizard mite, *G. texana,* is September 24, 1904, regardless of the notation "n.sp." which accompanied the description of 1906.

In his 1906 paper Banks noted that *G. texana* differed much from the other species of the genus *Geckobia* but did not give characters for separation. In 1904 and 1907 he placed this genus in the family ERYTHRAEIDAE (superfamily: TROMBIDOIDEA) along with the genera *Erythraeus, Anystis,* and another unnamed genus that apparently did not occur in the United States. In 1915 Banks revised his concepts of the families in the TROMBIDOIDEA and divided the ERYTHRAEIDAE into two families based on the structure of the chelicerae (chelate vs. non-chelate). The family ANYSTIDAE was erected for the four genera *Anystis, Tarsotomus, Pterygosoma,* and *Geckobia* which have chelate chelicerae. Banks stated here that species of the genus *Geckobia* occur only in Europe and placed the American lizard mite in the genus *Pterygosoma* Peters, 1849. According to this paper *P. texana* is the only lizard parasite of the family ANYSTIDAE in the Western Hemisphere.

Hirst (1917) removed the American lizard mite from the genus *Geckobia* and erected a new genus, *Geckobiella,* for it, with *G. texana* Banks as its type and only species. Hirst did not consider Banks' 1915 paper, as indicated by his reference to *Geckobia texana* and not to *Pterygosoma texana.* The name of the host was given as *Sceloporus spinosus* var. *clarkii* (= *S. floridanus*). No illustrations were presented. Location datum was Duval County, Texas. No mention was made of Banks' original location record of Travis County, Texas.

Hirst (1926) again included *G. texana* in his paper on the parasitic mites of the suborder PROSTIGMATA found on lizards. In this article the lizard mites are keyed to genera and species and many illustrations are presented. These drawings are a great improvement over the sketches by Banks. A brief description of the female of *G. texana* is given and the collection data noted as: Duval County, Texas, on *Sceloporus spinosus* var. *clarkii.* In this paper Hirst mentioned the lizard, *S. floridanus,* from which Banks' type specimens were taken.

Radford (1950a), in his list of mites parasitic on mammals, birds, and reptiles, noted that *G. texana* is the only species of *Geckobiella* and included the genus in the family PTERYGOSOMIDAE Oudemans, 1910. On page 388 of his host list, the host was given as *S. spinosus floridanus* but in his mite list, page 377, the host was given as *S. floridanus.* In two papers published in 1950, Radford (1950a, 1950b) gave the date of description as 1905 instead of 1904.

A review of publications shows that the only form of *G. texana* described is the adult female and that collection data and designation of host are confused. The purposes of this paper are to describe and illustrate all of the stages of *G. texana,* clarify the uncertain information concerning host, and add a new collection record.

TAXONOMY

The classification of *G. texana* is adapted from Baker and Wharton (1952).

Order: ACARINA Nitzsch, 1818

Suborder: Trombidiformes Reuter, 1909

Prostigmata Kramer, 1877

Family: Pterygosomidae Oudemans, 1910

Genus: *Geckobiella* Hirst, 1917

The genus is characterized in the description given of the female by Hirst (1917) as follows:

Body oval, longer than wide. Scutum absent. Many short plumose setae present on the dorsum and sides. Ventral surface with only a few simple setae. Tracheal horns long and directed anteriorly. Coxae divided into two groups, the first two pairs of legs being well separated from the last two pairs. Paired coxae not fused but separated by a well-defined suture, and bearing only simple setae. Type. *Geckobia texana* Banks, 1904. So far as is known this genus occurs only in the United States and includes only the type species.

Species: *Geckobiella texana* (Banks, 1904)

Synonyms: *Geckobia texana* (Banks, 1904)
Pterygosoma texana (Banks, 1915)

DESCRIPTION

Female (Plate 1D)

Body: (unengorged) Oval, longer than wide, transversely wrinkled, and without sclerotized plates except for minute platelets surrounding the bases of the ventral setae. One pair of lens-like eyes or eye spots, one on each side at about the level and just above the second pair of legs. Anal opening at posterior extremity. Genital structures located posteriorly on ventral surface, consist of two oblong longitudinal lobes laterad to the vulva each of which bear a single, long, simple seta on caudal margin. Internal arrangement of genitalia indistinct. Dorsal and lateral body setae (Plate 1F) plumose or scaly, very numerous on sides but scant in most of median portion of dorsum. Ventral setae relatively long and simple in structure except for the single barbed pair located just laterad to genital lobes. (Body more or less rectangular when engorged; related surface features distorted due to the distension.)

Gnathosoma: (Plate 1B) Conspicuous structure on cephalic margin wholly visible from dorsal and ventral views. Chelicerae long and stylet shaped, bearing small terminal segments that oppose the penultimate segments to form chelae. Chelae short, stout, without teeth or setae. Movable and immovable digits similar in size and shape. Base of each chelicera bulbous, expanding at about the mid-point to about six times the diameter of apical portion. Palps five segmented. Single pair of simple medio-lateral setae present on ventral surface of fused palpal coxae. Palpal segments two and three each bear a single, long, barbed seta dorsally. Fourth segment has one dorsal simple seta, a single lateral seta on each side, a strong, blunt apical claw, and a "palpal thumb" (the fifth segment) on inner lateral surface. Four simple, one barbed, and one transversely striated setae on palpal thumb. Rostrum (hypostome) truncate, toothless, and subequal in length to palpi. A pair of horn-like structures dorso-laterally, one on each side of bases of chelicerae, extend forward freely to level of mid-point of second palpal segment. These structures resemble internally the collar tracheae which are associated with species found in the mite family Tetranychidae and may be designated tracheal horns or peritremes.

Legs: First pair approximately as long as body length; remaining three pairs distinctly shorter. Coxal plates immovable, fused to ventral body wall, and separated on each side into two distinct groups. Lateral pairs of coxal plates distinctly separated and never fused into a single plate. Coxae I and II each bear two simple setae, one mesal and one distal. Coxa III bears three setae, one mesal simple seta and two distal setae, one of which is pectinate. Coxa IV is devoid of setae. All leg segments, except coxa, tubular in shape and about same thickness. Tarsi taper slightly at apices, but appear truncate. Tarsus I (Plate 1A) with anterior subapical hump which bears two setae, a shorter, thicker, transversely striated seta and a longer simple seta. Tarsus II bears a similar short, thick seta at the same location but lacks the accompanying simple seta. Tarsi III and IV (Plate 1E) without either of these distinctive setae. All tarsi have several pectinate setae and a pair of plumose setae. Paired tarsal claws, born on short pedicels, located distally on all tarsi. Each claw supports a double row of fine, filamentous, recurved hairs along outer margin. These hairs resemble closely the tenent hairs found on tarsal claws of spider mites (TETRANYCHIDAE). All remaining leg setae, except those noted on the coxae and tarsi, conspicuously pectinate.

Male (Plate 1C)

Body: Closely resembles female. Pyriform, longer than wide, widest at the level of the eye-spots. Integument finely wrinkled without sclerotized plates. Anal opening at caudal extremity. Visible genitalia consists of long, sharp, thorn-like structure pointing caudad and always protruding more or less from genital opening just below the anus. Associated reproductive apparatus indistinct.

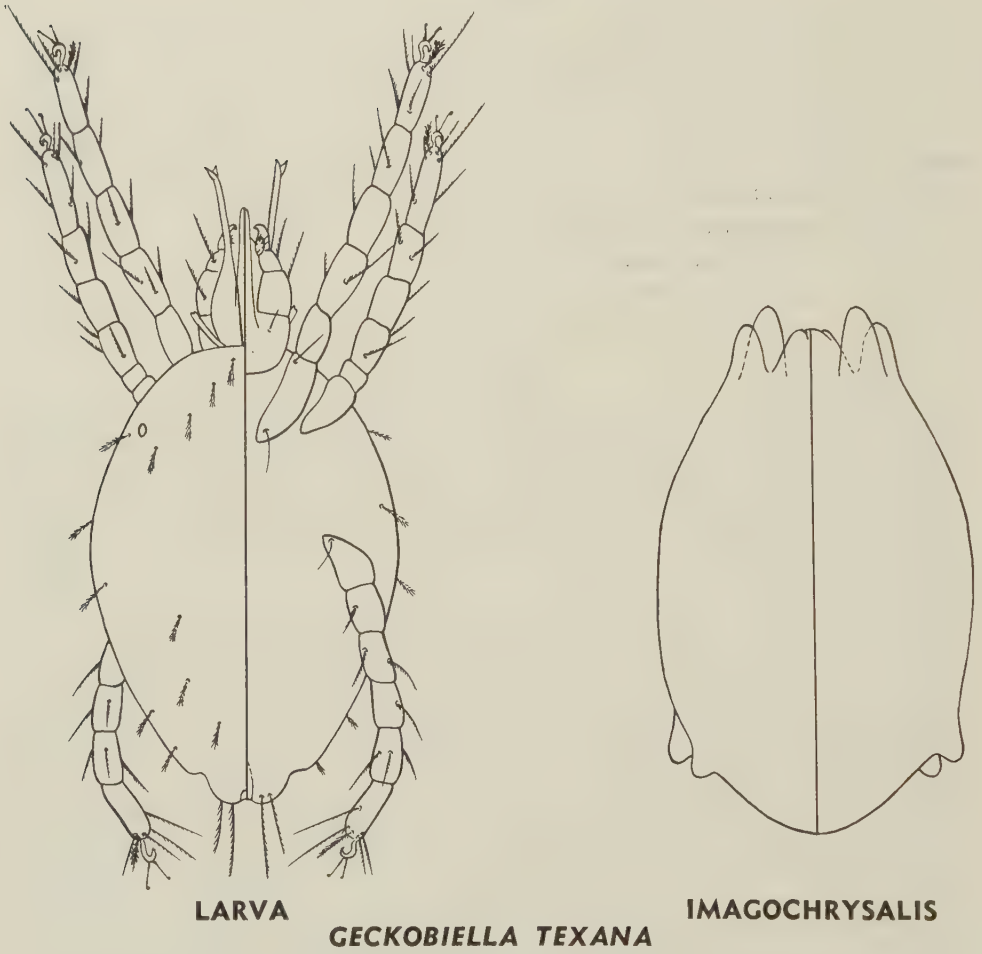

FIG. 1. *Geckobiella texana.* Larva and imagochrysalis.

Gnathosome: No structural differences discerned from female.

Legs: First pair conspicuously longer than body, exceed length of body plus gnathosome. Remaining three pairs shorter than body. Arrangement of coxal plates similar to female, lateral pairs appear to be closer together. Coxa I much larger than others, total area probably exceeds that of others combined. Coxae I almost meet along mid-ventral line. Setal pattern of the coxae identical to female. Remaining segmental setation of legs resemble female in all respects except for the stout, barbed, spine-like seta on the mid-ventral surface of patella IV.

Nymph (not figured)

Resembles female except for absence of genital structures.

Imagochrysalis (Fig. 1)

Oval, with a smooth hairless integument and nine protruding bud-like projections. Antero-median bud covers developing adult gnathosome and surrounding four antero-lateral buds encase developing fore legs. Postero-lateral buds similarly related to posterior two pairs of legs. The cast nymphal exuvia may occasionally be observed surrounding the imagochrysalis but there is no physiological connection. In latter stages the ensuing adult may be faintly visible through the integument.

Nymphochrysalis (not figured)

Resembles the structure of the imagochrysalis in all respects except for smaller size. (Table 1)

TABLE 1.—*Body measurements of* Geckobiella texana

Measurements in Millimeters	Total Length Body + Mouthparts			Body Length			Body Width		
	Max.	Min.	Avg.	Max.	Min.	Avg.	Max.	Min.	Avg.
Male	.75	.65	.70	.58	.50	.55	.50	.34	.37
Female*	1.05	.81	.91	.92	.68	.75	.63	.45	.51
Imagochrysalis	.95	.88	.90				.54	.51	.52
Nymph*	.70	.46	.53	.62	.35	.42	.30	.22	.25
Nymphochrysalis	.59	.53	.56				.37	.29	.34
Larva*	.31	.25	.26	.24	.18	.19	.17	.13	.14

* Unengorged.

Larva (Fig. 1)

Body: Shape similar to female. Setae few in number and relatively widely scattered over dorsal and lateral surfaces. Setae plumose as in adult. Ventral setae absent except for two pairs of long pectinate setae laterad to the anal opening. Pair of eye-spots at level of coxae II, one eye spot on each side of the dorsum. Genital structures absent.

Gnathosoma: Smaller, but identical in other respects to adult. Rostrum may be slightly longer than palpi.

Legs: All shorter than body length. Coxa I largest, bears two simple ventral setae. Coxa II without setae, coxa III bears one simple ventral seta. Leg setation same as female except that the short, thick, transversely striated setae of the first two pairs of legs are absent. Tarsal claws resemble those of adult but possess only a single pair of divergent tenent hairs on each claw.

Egg (not figured)

Ovoid, smooth, and white-toned in color. No other distinguishing characters are discernible.

DISCUSSION

Very little work has been done on the lizard mites of the family PTERYGOSOMIDAE other than that of Lawrence (1935, 1936), who described species on the lizard families GECKONIDAE, AGAMIDAE, ZONURIDAE, and GERRHOSAURIDAE in Africa, none of which occur prominently in the United States. GECKONIDAE is represented in this country by rare species in the most southern edges of California, Arizona, Texas, and Florida (Smith 1946).

According to Baker and Wharton (1952), eight genera are represented in the PTERYGOSOMIDAE. Of these, five are known to occur on various families of lizards in South Africa. *Geckobiella* occurs only on lizards of the genus *Sceloporus* (IGUANIDAE), which is restricted to the New World, and is reported only from Texas. This paper adds records of collections in Georgia from *S. undulatus undulatus* (Latreille).

ACKNOWLEDGMENT

Acknowledgment is made to Melvin H. Goodwin, Jr. who supplied material for this work, to Dr. Harry Pratt for encouragement and aid, and to Chester Stojanovich, Jr. who made the illustrations.

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PLATE I

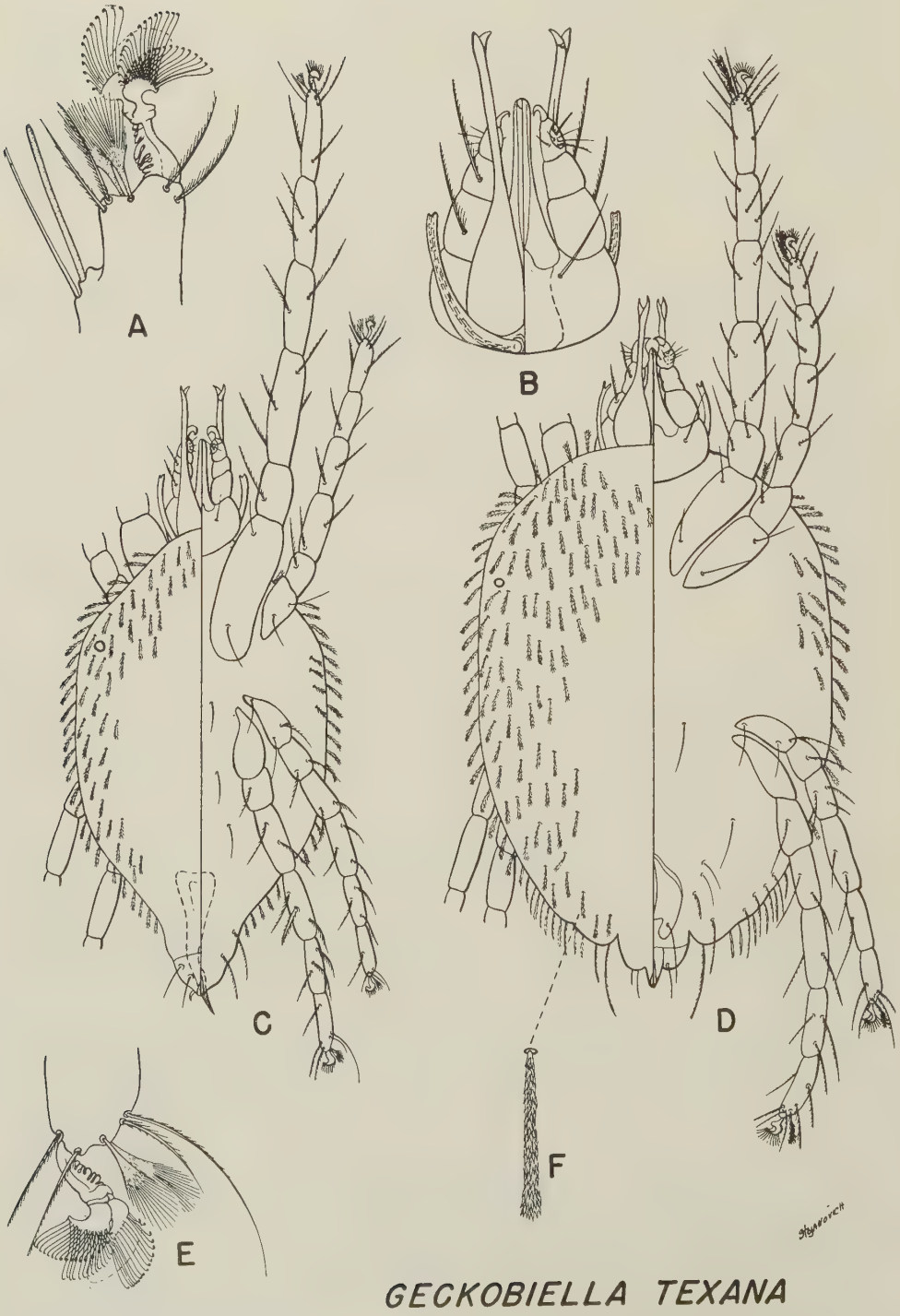

PLATE I. *Geckobiella texana.* A. tarsus I, B. gnathosome, C. adult male, D. adult female (unengorged), E. tarsus III, F. body seta.

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RESEARCH NOTES

RICKETTS AND TICK TRANSMISSION OF ROCKY MOUNTAIN SPOTTED FEVER

The classic studies of Dr. Howard T. Ricketts in the first decade of this century proved the transmission of the agent of Rocky Mountain spotted fever by what he called *Dermacentor occidentalis.* Under the title "Elk, winter ticks, and Rocky Mountain spotted fever," Philip and Kohls (Pub. Health Rep. **66:** 1672–1675, 1951) discussed a human case which was probably caused by a male of the one-host winter tick, *D. albipictus,* transferring from an Olympic Peninsula elk, and they commented that the undoubted use of this species in some of Ricketts' early experimental transmissions had thus become of more than academic interest. The question also was raised as to the possibility he might further have demonstrated natural infection in this species collected from horses in the Bitter Root Valley, if the unknown dates of collection had been in the winter time.

One of six fine copies of a "scrapbook" compiled by the family on the life and works of Dr. Ricketts was generously presented to the Rocky Mountain Laboratory by the widow of that eminent scientist before her recent death. It happened that the temperature records of two guinea pigs (numbers 596 and 622) in which he demonstrated natural tick infection were reproduced in the scrap book, but without the dates of collection of the ticks which were needed for deciding if they were winter or spring collections.

In the hope of finding a clue to whether they were *D. albipictus* or *D. andersoni,* Dr. Ricketts' son, Dr. Henry T. Ricketts, kindly examined his father's notes for us in regard to these tests, and supplied unpublished information that appears to be of both historical and factual importance to publish at this time. Guinea pig 596 was bitten on 5-22-07 by "36 males from horses near Hickory place" and became infected with spotted fever. Twenty-four of these were transferred and reattached to guinea pig 622 on 6-1-07, again with positive results. The actual dates of collection of the ticks are not stated. However, previous pages of his notes indicated the similar (but negative) testing of other ticks in April, taken from horses and cows on another ranch.

It appears, therefore, circumstantially evident that he would not have stored the male ticks until May if he had taken them off horses during the previous winter. Furthermore, if they had been *D. albipictus,* he would probably have at least noted the presence of nymphs on the same hosts, since other published notes which clearly concern that species have indicated his awareness of that stage of local ticks.

It is deduced in consequence, that while Ricketts undoubtedly did show transstadial transmission by some experimentally infected *D. albipictus* (originally winter collected as nymphs off Bitter Root horses), the positive, natural infection tests were performed above with spring collected *D. andersoni,* the customary Rocky Mountain vector to man. In the main, his experimental transmissions were accomplished with this last species, as later elaborated with Maver.

It is pertinent also to redirect attention to his comments in 1909 (J. Amer. Med. Assoc., **52:** 539) on "a micro-organism which apparently has a specific relationship to Rocky Mountain spotted fever" in which he more than vaguely describes Giemsa staining "diplococcoid bodies" of some pleomorphism which he observed in vertebrate blood, but especially in the eggs and tissues of infected (but not in uninfected) ticks. Agglutination tests further convinced him of the probable specific etiologic relationship of these bodies, but in view in part of his now understandable failure to cultivate them, he "devised no formal name for the organism discussed." Wolbach supplied this later after da Rocha Lima had so ably clarified the status of and named the agent of epidemic typhus. da Rocha Lima lost sight of these earlier studies in assaying

Ricketts' similar observations on the typhus agent, when he recently remarked at length (Revista Brazileira de Medicina, **8**: 312–320, 1951) that "the findings of Ricketts were not and could not have been, confirmed as a discovery" (translation).

As far as the writer knows, paying justified homage to Ricketts' original sound and discerning investigations has involved no lessening of esteem among North American scientists for the essential work of da Rocha Lima. The observations of both men were landmarks in pioneer discoveries of this group of diseases and both are justly given their respective shares of credit.—Cornelius B. Philip.

NATURAL INFECTIONS OF *FASCIOLOIDES MAGNA* IN WISCONSIN SHEEP

Swales (Can. J. Research, **12**: 177–215, 1935) reported that *Fascioloides magna* (Bassi, 1875), the giant liver fluke of deer, reached maturity in sheep, following experimental exposure to encysted cercariae. He presumed that *F. magna* would also reach maturity in naturally incurred infections in sheep. During the period March through June, 1953, three instances of mature, natural infections of *F. magna* were recorded by the present authors in sheep from northern Wisconsin. In each case the infection had proved fatal. In one case the liver contained five flukes, of which the largest measured 6.6 cm. × 3.1 cm.

That at least some of the flukes in each infection had reached sexual maturity was shown by the collection of mature ova either from the bile or the uterus of the flukes. The presence of ova in the bile would seem to confirm Swales' contention that sheep, unlike cattle, play a role in the dissemination of this parasite similar to that played by the normal definitive hosts, (Cervidae).—W. C. Campbell and A. C. Todd, *Dept. Veterinary Science, University of Wisconsin.* Paper No. N.S. 145. Published with the approval of the director of the Wis. Agric. Expt. Sta.

THE MAINTENANCE OF *TRICHOMONAS VAGINALIS* ON A SOLID MEDIUM

This note is to report the successful axenic maintenance of the protozoan parasite, *Trichomonas vaginalis,* on Bacto NIH agar without subculturing for a period of 23 days. The technique is as follows: 0.2 ml of a 48 hour culture of trichomonads in CPLM medium (Johnson and Trussell, Proc. Soc. Exp. Biol. and Med. 54: 245–249; 1943) is overlayed on a slant of Bacto NIH agar; the cotton plug in the tube is sealed over with Parafilm and the tube is placed in a 37° C. incubator. Within 48 hours an opaque white line appears at the interface of the slant and air space. Removal of material from this region with a wire loop reveals no active trichomonads for periods of up to 20 days. Flooding of the culture slant on the 20th day with 2.0 ml. of CPLM medium produces, by the 23rd day, a very abundant growth of *Trichomonas vaginalis* which are normal in appearance and very active. Cultures were discarded on the 25th day.

The maintenance of *Trichomonas vaginalis* on slants for this length of time should afford greater opportunity for more widespread study, since one of the greatest problems involved in working with this organism has been the need for frequent transfers in a liquid culture medium. —Samuel K. Wirtschafter, *Department of Zoology, University of California at Los Angeles.*

GIANT MULTINUCLEATED CELLS IN CULTURES OF *TRICHOMONAS VAGINALIS*

Since as early as 1894 (Marchand; Zentrb. Bakt., 15: 709–720) large multinucleated individuals of *Trichomonas vaginalis* have been reported. Kofoid and Swezy (Proc. Amer. Acad. Arts and Sci., **51**: 289–378; 1915), reported the existence of large cells containing up to 8 nuclei in *Trichomonas augusta, Trichomonas muris, Eutrichomonas serpentis,* and *Tetratrichomonas prowazeki.* These multinucleated forms were given the name somatella. These somatella or syncytial forms contain a full complement of organelles. Giant somatella individuals have been noted in recent studies involving *Trichomonas vaginalis.*

It has been possible to induce the formation of large populations of these multinucleated cells by the simple process of increasing oxygen tension. The procedure followed is here outlined: 1.0 ml. of a 48-hour axenic culture of *Trichomonas* grown in CPLM medium (Johnson and Trussell, Proc. Soc. Exp. Biol. and Med., **54**: 245–249; 1943), is inoculated into a flask containing 100 ml. of CPLM medium in which the agar has been deleted. The culture is incubated for 48 hours at 37° C. Flask cultures which are not placed within an evacuated desiccation chamber show an oxidized state with reference to methylene blue indicator. These cultures show a very high proportion (50%–80%) of giant somatella trichomonads. Giant cells subcultured and incubated under reduced oxygen tension produce, in 48 hours, cultures which are completely normal and without giant cells. This procedure for production of somatella cells from normal trichomonads and the reverse procedure may be carried on indefinitely.

Giant cells were examined in the living state, with a phase microscope, and in the fixed condition on permanent iron hematoxylin slides. In both types of preparations the existence of the polynucleated state and the existence of the duplicated condition of the organelles were easily seen.

It appears that oxygen tension inhibits cytoplasmic division in the anaerobic parasitic protozoan, *Trichomonas vaginalis*. This view is favored in preference to that of increased nuclear and cytoplasmic division, since the populations in cultures containing somatella forms are smaller.—SAMUEL K. WIRTSCHAFTER, *Department of Zoology, University of California at Los Angeles.*

OBSERVATIONS ON THE BIOLOGY OF *ACETODEXTRA AMIURI* (STAFFORD, 1900) PEARSE, 1924, IN LAKE ERIE

Incidental to other investigations, considerable information has been obtained concerning the biology of *Acetodextra amiuri* in Lake Erie fishes. This distome is apparently widely distributed in the Mississippi and St. Lawrence watersheds (Stafford, 1900, Pearse, 1924, Bangham and Hunter, 1939, and Perkins, 1950), and in Lake Erie it has been recovered either from the air bladders or ovaries in specimens of several species representing two families of fishes. In the course of casual observations, it was found in *Ameiurus nebulosus, A. natalis, A. melas, Noturus flavus,* and *Cottus bairdii.* However, a more complete study of this worm was made in the course of the examination of 94 specimens of *Ictalurus lacustris.* In this fish the level of infection was low, with the largest number of worms being 10 necrotic specimens in the ovary of a host 32 cm. long. However, the average infection for each host was between two and three, while the median was only one. The percentage of infection in this species of fish was 11.7. The specimens found in this study were, as a rule, necrotic; of 28 worms recovered, in the case of 24 of them, the distome's body had decomposed leaving a mass of eggs. Of these egg masses, 13 were recovered from the ovaries and the rest were found in the air bladders. The incidence of infection recorded above was not altered significantly through the subsequent examination of hundreds of ovaries obtained from a commercial fish house in the area.

Perkins, 1950, did not mention the occurrence of this trematode in the air bladder, stating rather that the ovary is its natural habitat. In Lake Erie fishes, it appears that both the ovary and air bladder may serve as the definitive location. In either location, it appears that the eggs remain in the worm's uterus which persists for some time. It was observed that the adult had completely decomposed in most cases. When this occurs in the air bladder, only a patch of eggs, which is embedded in the mucous, remains. From the air bladder, the eggs probably leave the host via the *ductus pneumaticus* and the alimentary tract. However, the *ductus pneumaticus* is not ciliated and an extract of intestinal enzymes of *I. lacustris* did not elicit hatching of the eggs. The "clumps" of eggs in the ovary are probably expelled from the fish at the time of spawning.

Perkins, 1950, described an adaptation for egg dispersal in which *A. amiuri* ruptures the uterus and expels the eggs by muscular contraction. The small number of living worms found in the ovary of fishes of this locality precludes the possibility of these (living) worms playing any great role in the dispersal of eggs in the manner described by Perkins.

Eggs containing fully developed miracidia were recovered from ovaries of one specimen of *Cottus bairdii.* When placed in the body "juices" of *Campeloma decisum,* these eggs hatched in less than a minute. The miracidia on release swim slowly in close spirals. Attempts to fix the miracidia without their being distorted were unsuccessful.—WILLIAM H. COIL, *Franz Theodore Stone Institute of Hydrobiology, The Ohio State University.*

TWO CASES OF APPARENT MYIASIS BY *MUSCA DOMESTICA* LINNAEUS IN THE GUINEA PIG

Two instances of apparent myiasis in the guinea pig caused by the common domestic housefly, *Musca domestica* Linnaeus, were discovered by one of us (J.K.F.) at autopsy in 2 guinea pigs on the same day. The guinea pigs were of normal stock kept in an animal house colony of the National Institutes of Health, Bethesda, Maryland. Many cases of streptococcal lymphadenitis and otitis media were observed in the colony at the time. The 2 animals in question had died in the colony, had been removed to a refrigerator, and had been brought to autopsy all within probably less than 24 hours. Postmortem invasion in each instance is unlikely since most of the larvae were mature and pupated the day after removal from the host. The larvae were removed on March 14, 1951. All adults that emerged from pupae which had formed by the next day and all remaining larvae were determined by one of us (P.A.W.) to be *Musca domestica* Linnaeus. The larvae and adults were normal in size.

Three mature larvae were found in the left middle ear cavity of the first animal. The following morning 2 of the 3 were dead. The third had pupated. A female adult emerged from the pupa. It appeared possible that the larvae had entered via an ear drum that may have ruptured secondary to streptococcal otitis media. However, this could not be definitely ascertained.

Twelve third stage larvae were recovered from the "scrotal-perineal sac" of the second animal. The following morning, of the 7 mature larvae which had been kept under observation, 5 had become pupae. Three adult females and 1 adult male emerged from 4 of the pupae; the fly of the fifth pupa was lost before the sex was noted. As sometimes occurs in male guinea pigs, fecal material had accumulated in the sac where it had become concreted and had caused ulceration of the skin. No intestinal myiasis was found in this animal.

Apparently authentic human cases of enteric myiasis, urinary myiasis, and auricular myiasis, by *Musca domestica,* have been reported (James, U. S. Dept. Agric. Misc. Publ. No. 631, 1947). —Paul A. Woke, *National Institutes of Health, Bethesda, Md.,* and J. K. Frenkel, *School of Medicine, Univ. of Kansas, Kansas City, Kansas.*

THE INACTIVITY OF CERTAIN 6-HYDROXY-8-AMINOQUINOLINES IN EXPERIMENTAL CHAGAS' DISEASE

A number of 6-methoxy-8-aminoquinolines of the type which have been found to be useful curative drugs in vivax malaria also have chemotherapeutic activity against *Trypanosoma cruzi,* the causative agent of Chagas' disease (Goble, 1952, Antibiotics and Chemotherapy, 2: 265). Other studies have indicated that the demethylation of compounds of this type reduces antimalarial activity along with toxicity (Greenberg, Taylor and Josephson, 1951, J. Inf. Dis. 88: 163). When the 8-amino-6-quinolinol "Certuna" (SN-191) was found to be without activity against *T. cruzi* (Goble, 1949, J. Parasitol. 35: 375), the testing of two additional quinolines seemed desirable.

The compounds selected were the demethylated derivatives of pentaquine (Drake et al., 1946, J. Am. Chem. Soc., 68: 1529) and pamaquine (Steck and Boehme, 1952, J. Am. Chem. Soc., 74: 4511). Both of the 6-methoxy parent compounds had readily demonstrable activity against *T. cruzi* infections in mice at dose levels of 15 mg./kg./day (Goble, 1949, 1952, references above). The two 6-quinolinols (DR-15324 and WIN-3425 respectively) were tested, therefore in comparison with the corresponding 6-methoxyquinolines at that dose level; the animals were medicated once daily *per os,* by intubation, 6 days a week for 3 weeks. The demethylated compounds had no influence on the course of the disease in either of two tests which were conducted. —Frans C. Goble, *Charlotte Amalie, St. Thomas, U. S. Virgin Islands.*

AN ADDITION TO THE TEXAS FLEA FAUNA

A flea not previously reported from Texas has been taken during an ectoparasite survey of Wichita and adjacent Counties in north-central Texas. A single male specimen of *Malaraeus sinomus* (Jordan) was taken from six deer mice, *Peromyscus boylii,* trapped three miles south east of Lazare, in Hardeman County, Texas, June 27, 1953, by W. W. Dalquest. The Lazare locality represents the extreme eastern range of *P. boylii.* The only other ectoparasites were two *Haemolaelaps glasgowi* mites.

This flea was compared with a *Malaraeus sinomus* male from Riverside County, California, which was included in a good series of fleas presented the Texas State Department of Health by Dr. G. F. Augustson. No specific differences between the Texas and California specimens were noted. This is the first species of the genus *Malaraeus* recorded from Texas.

Texas must represent the eastern limit of the distribution of this flea, since numerous *Peromyscus* have been examined in the state, with this single recovery.—R. B. Eads, *Texas State Department of Health, Austin,* and W. W. Dalquest, *Midwestern University, Wichita Falls, Texas.*

NOTES ON *MONILIFORMIS CLARKI* (WARD) IN EASTERN NEW YORK (MONILIFORMIDAE: ACANTHOCEPHALA)

The large acanthocephalan, *Moniliformis clarki* (Ward) has been reported from several mammals (Van Cleave, 1953: Illinois Biol. Monog. **23**: 130–131). Although it is characteristically found in squirrels and chipmunks (eleven of eighteen recorded occurrences are from sciurids), it was found by the author to be common in the small intestine of the northern pine mouse, *Pitymys pinetorum scalopsoides,* at Kingston, Ulster County, New York. This record was cited by Van Cleave (*loc. cit.*) on the basis of specimens sent to him by Dr. B. V. Travis of Cornell University. Attention is called to another host record of *M. clarki* omitted by Van Cleave, that of Doran and Read (1951, J. Parasit. **37** [3]: 330), who collected this species from the southern grasshopper mouse, *Onychomys torridus,* in California.

Adults of this species were most commonly found during the summer and autumn months. About half of 28 pine mice taken in October, 1951 and July, 1952 were infested. Only one of seven pine mice taken at the same spot in late April, 1952 was infested. Although this species of worm may reach 400 mm. in length, all specimens from the pine mice were less than 200 mm. The total length of the small intestine in the pine mouse is 200 to 250 mm., so that some specimens of the parasite extend for virtually its entire length. Despite the small size of the host (120–130 mm. in length, 20–30 gms. in weight), the parasite may be present in considerable numbers. Twenty-two worms were removed from one animal, in which the small intestine was so packed with worms that it is difficult to imagine how normal digestive processes could have taken place.

Several meadow mice, *Microtus p. pennsylvanicus,* from the same locality were infested with *M. clarki,* which is a new host record for this species. A more typical host in this area, judging by previous records, is the chipmunk, *Tamias striatus lysteri.* A chipmunk taken on June 7, 1953, near Mount Tremper, about 20 miles west of Kingston, had one large *M. clarki* in the small intestine.

I am indebted to the late Dr. Harley Van Cleave, who identified the first collection of this species from Kingston.—Allen H. Benton, *Department of Biology, New York State College for Teachers, Albany, New York.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Forty-third Council Meeting, Madison, Wisconsin, September 7, 1953

The meeting of the Council of the American Society of Parasitologists was called to order by President-Elect Elery R. Becker at 8:00 P.M., September 7, 1953, in a recreation room of Elizabeth Waters Hall of the University of Wisconsin. In the absence of President Philip, President-Elect Becker served as presiding officer for the Madison Meetings. Past Presidents W. W. Cort, G. R. LaRue, and E. W. Price, and the following members of the Council were present: P. C. Beaver, C. A. Herrick, A. McIntosh, D. B. McMullen, D. V. Moore, R. M. Stabler, N. R. Stoll, H. W. Stunkard, J. C. Swartzwelder, A. C. Walton, and G. W. Wharton.

The regular order of business was taken up.

I. Reports of Officers and Members of Council

1. *President (C. B. Philip):* No report. Dr. Philip sent his good wishes to the Council and regretted that attendance at International Congresses in Copenhagen and in Rome prevented his being present at Madison.

2. *Vice-President (H. W. Brown):* No report. Dr. Brown sent his regrets that he was unable to attend the Madison Meetings.

3. *Secretary (A. C. Walton):* As of September 7, 1953, the Secretary's record shows that there were 851 members of the Society, of whom 95 live outside of the country and 756 live within the United States (25 are temporarily on foreign assignments). Of these 851 members, 1 is listed as a Life Member, 12 are Foreign Honorary Members, and 838 are Active Members. Of the 838, 765 are in good standing, 35 are delinquent for one year, and 38 are delinquent for two years and will be dropped as of January 1, 1954, unless paid up before that date. During the year 53 were elected to active membership, 1 to life membership, and 4 to honorary membership. Later in the meeting the names of 19 additional persons will be presented to the Council for election to active membership.

During the year notices of the death of two Charter Members of the Society reached this office (Dr. H. E. Ewing and Dr. H. J. Van Cleave). The resignation of 17 members, including 1 Charter Member were received, and 34 were dropped for non-payment of dues.

The office of the Secretary received $200.00 for incidental expenses during the fiscal year and attached receipts indicated expenditures of $193.89. The last fiscal year ended with a balance as of September 7, 1953, of $36.85. Anticipated bills for the balance of the year are well within the limits of this amount (*N. B.* Local Committee expenses totaled $6.90).

The Secretary's report was accepted subject to audit and ordered placed on file.

4. *Treasurer (R. M. Stabler):* An interim report of the funds under the control of the Treasurer was presented. A summary for the period, September 1, 1952 to September 1, 1953 follows:

a. Balance on hand as of September 1, 1952 was $6,461.72.

b. Expenditures September 1, 1952 to November 1, 1952 were $1,647.19.

c. Balance on hand as of November 1, 1952 was $4,814.53 (end of fiscal year).
d. Collections from all sources to September 1, 1953 were $11,875.13.
e. Total funds available, therefore, were $16,689.66.
f. Total funds expended since November 1, 1952 were $9,863.87.
g. Balance on hand as of September 1, 1953 was $6,825.79.

The Treasurer further reported that the completed report for the fiscal year ending last November 1, 1952, had been audited by the committee appointed by the President and is now on file with the Secretary, certified as correct, and has been published in the Journal Supplement of this year.

The interim report was accepted and ordered placed on file. The Treasurer was instructed to send the final report as of November 1, 1953, to the members of the special auditing committee (A. McIntosh and E. W. Price) and when certified as correct, to be incorporated in the minutes of the Society as accepted.

5. *Custodian of the Endowment Fund (N. R. Stoll):* The Custodian's report to the Council for the period of the fiscal year ending November 1, 1952 showed a balance of $1,113.46—the same as the interim report of last September. The interim report for the period November 1, 1952 to July 6, 1953 shows a balance of $1,154.21, and consists of a savings account of $387.21 and a U.S. savings bond of $1,000.00 face value issued January, 1950 and carried on the books at its current redemption value of $767.00. Ex-officio trustees for the current fiscal year are C. B. Philip, President, and R. M. Stabler, Treasurer, of the Society. The Custodian bespeaks the interest of those who believe in parasitology (including parasitologists themselves) in remembering and befriending the Endowment Fund.

The final report of the fiscal year ending November 1, 1952 was presented for audit and when certified by the auditing committee is to be accepted and placed on file. The interim report for the current year was accepted as read and ordered placed on file. Final report of the Custodian will be audited by the special auditing committee after November 1.

6. *Custodian of Back Issues (G. F. Otto):* No report but Dr. Otto sent greetings to the group from abroad where he is attending international scientific meetings. (*N. B.* Dr. Otto's report, now at hand, is as follows and is appended to the minutes as audited by the special auditing committee.)

Financial Report of the Custodian of Back Issues, Aug. 31, 1952 to Sept. 30, 1953.

Credits:

Balance on hand August 31, 1952		$705.89	
Accounts collected:			
Regular issues	$2,284.40		
Portraits	16.25		
25-volume indices	78.50		
		$2,379.15	
			$3,085.04

Debits:

Duplication of back issues	$1,781.03		
Insurance on Journal stock	36.25		
Shipping labels	12.25		
Refund of overpayment	.50		
Postage and express	96.41		
Clerical help	25.00	$1,951.84	
Balance on hand Sept. 30, 1953		1,133.20	
(Expense vouchers submitted for audit)			$3,085.04
(Bank Statement as to balance submitted for audit)			

1952 Accounts carried forward

Accounts collectible:

Portraits		$3.50	
25-volume indices		2.05	
Regular issues	$1.75		
	4.00		
	12.15		
		$17.90	$23.45

Debits:

Shipping boxes	$53.04		
Collection for J. W. Scott	250.00		$303.04
Shipping Journals to W. S. Bailey (estimate)			$500.00
New shelving at A. P. I. (estimate)			100.00
			$903.04
Loan from the American Chemical Foundation			
Due Nov. 29, 1953		$650.00	
Due Jan. 4, 1954		1,850.00	
			$2,500.00
(Application for time extension pending)			
Current status of 25-volume index			
Original cost of 1,200 copies			$1,292.72
Collections on sales prior to 1953		$1,158.85	
Collected during 1953 ($131.25 less $8.00 postage expense)		123.25	
			$1,282.10
Investment not yet liquidated			$10.62
Copies available for sale	374		
September 30, 1953	Signed	G. F. Otto, Custodian	

7. Chairman of the Editorial Committee (H. W. Stunkard): The interim report of the Committee covers the October and December numbers of Volume 38 (1952) and the first four numbers and August Supplement of Volume 39 (1953). Volume 38 complete, contained 661 pages; contained 83 research articles, numerous Research Notes, other pertinent material; included a Supplement of 51 pages; and cost $10,561.34. The first four numbers of Volume 39 contained 466 pages of research material (69 articles and 12 Research Notes) and the Supplement contained 48 pages, and cost $7,549.61. In spite of increased costs of paper and of printer's services, careful supervision and a slight increase in advertising rates has kept the cost approximately the same per page as they were last year ($14.46). 26 papers and 29 Research Notes are in galley proof and only 4 papers are in the hands of authors for correction. In addition, 19 papers are in the hands of authors for revision pending being placed in galley. In general, papers have appeared within 6 months after reaching galley stage.

Inasmuch as the Editor and his Committee end their five-year term of office with the completion of Volume 39, the Editor summarized the activities and policies utilized during that term and expressed personal as well as Committee appreciation to all of those who have assisted the work during that time. Special appreciation is expressed for the services of Dr. Cort—who acted as Editor for the year 1948 during the sabbatical leave of Dr. Stunkard—and to the Press and to its manager, Mr. George M. Houck.

For the information of the membership of the Society, Dr. Stunkard presented a summary of the Journal expenses during his entire term of service of 10 years. A detailed report is on file in the Secretary's office.

Year	Volume	Pages published	Copies printed			Cost
1944	30	400 + 24 in Suppl.	Nos.	1–3	1100	
				4–6	1200	$3,320.50
			Suppl.		1200	
1945	31	456 + 32 in Suppl.	No.	1	1350	
				2–4	1300	
				5–6	1400	$4,235.21
			Suppl.		1400	
1946	32	608 + 26 in Suppl.	Nos.	1–6	1400	$5,682.19
			Suppl.		1550	
1947	33	530 + 48 in Suppl.	No.	1	1450	
				2–6	1600	$5,550.73
			Suppl.		1600	
1948	34	529 + 44 in Suppl.	No.	1	1700	
				2–6	1650	$7,638.69
			Suppl.		1725	
1949	35	639 + 52 in Suppl.	Nos.	1–6	1700	$9,032.12
			Suppl.		1800	

1950	36	616	+58 in Suppl.	Nos. 1–6	1900	$9,020.28
				Suppl.	2000	
1951	37	609	+59 in Suppl.	Nos. 1–6	1900	$9,418.90
				Suppl.	2000	
1952	38	610	+51 in Suppl.	Nos. 1–6	1900	$9,808.39
				Suppl.	2000	
1953	39	693	+48 in Suppl.	Nos. 1–6	2000	$11,124.64
				Suppl.	2100	

The complete report was accepted with a vote of thanks to Dr. Stunkard and his Committee and ordered placed on file.

At this time Dr. Cort presented the following Resolution *re* Dr. Stunkard:

"The Council of the American Society of Parasitologists regrets the retirement of Dr. Horace W. Stunkard from the position as Chairman of the Editorial Committee of the Journal of Parasitology, which he has held since 1944. It wishes to call to the attention of the Society his unusual record of services in this and other positions.

Dr. Stunkard was secretary of the Society from 1933 to 1937. His careful attention to detail, his ability to plan ahead, and his qualities of leadership made him an outstanding success in this position. Because of his high standing in the field of parasitology he was elected to the presidency of the Society in 1939. From 1940 to 1943 he served as a member of the Council, and in 1944 he accepted the duties of Chairman of the Editorial Committee.

In spite of a crowded program of administration, teaching, and research in his regular position, Dr. Stunkard has handled very effectively every phase of the work involved in the position of Chairman of the Editorial Committee. He has shown marked business ability in his dealings with the Press, and in the handling of the financial affairs of the Journal. With real imagination and a tempered optimism he has planned ahead, and has steered the Journal successfully through the uncertainties of the post-war period. At critical times he has, through his own efforts, obtained grants from outside sources to enable the Journal to meet more adequately the increasing demands for publication.

He has set high standards for the papers he has accepted for publication, and has encouraged authors to meet these standards by giving them detailed constructive suggestions on writing and organizing material, both from his own study of their papers and from suggestions made by other reviewers. He has been particularly successful in utilizing the help of members of the Editorial Board and other specialists in reviewing the large number of papers submitted for publication in the Journal. In addition, he has interested himself in the broader problems of scientific publication, and has had a leading part in shaping the general policies of both the Journal and the Society.

The Council wishes to express to Dr. Stunkard its thanks for the important services he has rendered. It acknowledges that it is now time for him to be relieved of the burdens of detailed editorial work, but it is looking forward to his help and guidance for many years to come in the affairs of the Journal and Society."

II. Reports of Standing Committees

1. *Committee on Visual Education and Instruction (M. S. Ferguson, Chr.):* The committee reports that it has prepared a mimeographed compilation "Visual Aids to Instruction in Parasitology," a copy of which is appended, and that copies are available at the meeting for all of those interested. Further inquiries may be addressed to the chairman at the CDC Mycology Laboratory, Chamblee, Ga. It was hoped that the committee be continued and that additional material may be added to the compilation during the coming year.

The report was accepted and placed on file and it was voted to continue the committee as constituted for another year.

2. *Committee on the Terminology of Avian Strains of Malaria (C. G. Huff, Chr.):* The committee reported that the need for such a group has largely ceased and that they recommended that the committee be discharged. Dr. Huff offered to serve in an unofficial capacity in case any need should arise during the coming year.

The report was accepted and ordered placed on file with the thanks of the Council for a work well done. The Committee was discontinued and the offer of Dr. Huff to act in an unofficial capacity was accepted with appreciation for his offer.

3. *Committee on Common Names of Helminths (P. D. Harwood, Chr.):* The committee reported the preparation of the list as accepted last year and its publication in the Journal. The committee felt that its major efforts were completed and that there was no need for its continuation. Dr. Harwood offered to continue his services in an unofficial capacity for the next year.

The report was accepted with thanks and ordered placed on file. The committee was discontinued, but the offer of Dr. Harwood to act unofficially was received with appreciation.

4. *Auditing Committee Reports (A. McIntosh and D. V. Moore):* The committee audited the financial reports of the Secretary and of the Custodian of Endowment Funds and found them correct and in order. A special committee of A. McIntosh and E. W. Price will audit the final reports of the Treasurer, of the Custodian of Endowment Funds, and of the Custodian of Back Issues when those are presented at the end of the fiscal year (November 1, 1953) and report to the Council.

III. Reports of Special Committees

1. Dr. G. R. LaRue presented a resolution in memory of Dr. H. J. Van Cleave as follows:

"Whereas on the second day of January 1953 death has removed from the membership of the American Society of Parasitologists Harley Jones Van Cleave, a charter member who since its inception has given to this Society his constant and loyal support by participation in its meetings and further by his services to the Society as a member of the Council for the years 1938 to 1941, as its Vice-President in 1935, and as its President for the year 1947; and

Whereas Dr. Van Cleave throughout his long career as a teacher and as an investigator has strongly stimulated many young men and young women to scholarly pursuits; and

Whereas the members of this Society have lost a considerate friend and wise counselor; therefore

Be it resolved that the American Society of Parasitologists assembled in regular annual meeting in the City of Madison, Wisconsin, on this ninth day of September, 1953, hereby express its sense of profound sorrow and great loss in the death of Harley Jones Van Cleave; and

Be it further resolved that these resolutions be entered in the permanent records of this Society, and that copies be transmitted to his widow and to the members of the family."

The resolution was accepted by a standing vote, ordered to be read before the annual meeting, spread on the records of the Society, and the Secretary empowered to carry out the directions concerning sending the resolution to those concerned.

2. *Committee on selecting nominees for the New Editorial Committee to handle the Journal beginning with Volume 40 (1954)—(N. R. Stoll, Chr.):* The committee reported that after canvassing the situation for a number of months and consulting all former members of the Editorial Committee and the Editorial Board, they were presenting the name of G. R. LaRue as their nominee for Chairman of the Editorial Committee and the names of C. G. Huff and C. B. Philip as the other two members of the Committee, thus giving representation on the Committee to a helminthologist, a protozoologist, and an entomologist, as prescribed by the Constitution.

The Committee was thanked for their intensive study of the situation and the Council ordered that the names of the three men so recommended be presented to the Society at the annual meeting as elected to those respective positions.

3. *Committee on the selection of a nominee for the position of Custodian of Back Issues (Council as a Committee of the Whole):*

a. The Council accepted with regret the resignation of Dr. G. F. Otto as Custodian of Back Issues before the end of his term due to his change of residence, and ordered the following resolution prepared by Dr. Cort to be presented to the Society and to be placed in the files:

"The Council of the American Society of Parasitologists accepts with regret the resignation of Dr. Gilbert F. Otto from the position of Custodian of Back Numbers of the Journal of Parasitology. It wishes to call to the attention of the Society the very effective service that he has rendered in this position.

Dr. Otto served as treasurer of the Society from 1937 to 1940, and again in 1944. During this period he became interested in trying to expand the sale of back numbers of the Journal of Parasitology. He also developed the idea of duplicating the missing issues, and in 1945 he took over this work as an unofficial representative of the Treasurer. By 1948 the business had grown to such an extent that he was elected to the newly created position of Custodian of Back Numbers. In this same year the Council voted $1,500.00 of general funds to begin the duplication of missing issues. Such a demand developed that in 1950 the Custodian was authorized to borrow $3,000.00 from the American Chemical Foundation. This money was interest-free, and, added to the money obtained from the greatly enlarged sale of back numbers, has made possible the purchase of 200 copies each of more than 30 missing issues. The annual sale of back numbers has increased over this period from about $400.00 a year to nearly $4,000.00. After the few issues that are still missing have been duplicated, the Society will have a substantial source of income from the sale of back numbers that will continue for many years. Also, the availability of complete volumes and sets of the Journal of Parasitology for libraries and individuals has been a real service to parasitologists.

The Council wishes to express to Dr. Otto its thanks for these untiring efforts by which, over so many years, he has rendered an outstanding service to the Society."

b. The Council, acting as a committee of the whole, then agreed to accept the name of Dr. W. S. Bailey of the Alabama Polytechnic Institute, Auburn, Alabama, as their nominee for the unexpired term of Dr. Otto and as the new Custodian for a three-year term beginning January 1, 1954 (Dr. Bailey has indicated his willingness to accept the duties and that his Institution has the necessary storage space for the bulky issues now in hand and also for those still to be duplicated), and to report to the Society that he has been elected to the office of Custodian.

IV. Reports of Representatives and Delegates

1. *To Council of A.A.A.S. (A. O. Foster and K. C. Kates):* The Representatives presented the Minutes of the Council of the A.A.A.S. meeting held at St. Louis in December, 1952. Of interest to our Society was the report of the election of Dr. Warren Weaver as President-Elect of the Association and of Dr. Paul Weiss as Vice-President in charge of Section F (Zoology). Letters concerning the actions which led to the resignations last March of Dr. Meyerhoff as Administrative Secretary of the A.A.A.S., and of Mrs. Keener as Editor of the A.A.A.S. Journals were also appended to the report.

The report was accepted and ordered placed on file.

2. *To Governing Board of A.I.B.S. (W. W. Cort):* Dr. Cort reported on the activities of the A.I.B.S. The Institute now consists of 17 member Societies with a membership of over 12,000 scientists, a staff of 10, and an annual budget of over $50,000.00. Besides sponsoring the annual September meetings and publishing the Bulletin and Annual Program number, the Institute is active in a number of projects: viz., Bio-Sciences News Letter to foreign countries, a Handbook of Biological Data, a Scientific Register, a Placement Service, Vocational Guidance literature, a textbook survey, and the Institute of Animal Resources—in addition to many other less easily defined problems confronting Biologists in general at this time. One such problem has to do with a proposed centralization of the handling of business affairs of the Journals of the various member Societies in the A.I.B.S.

The report was accepted and ordered placed on file.

3. *To the Division of Biology and Agriculture of the National Research Council (A. C. Walton):* Dr. Walton reported that the meeting of April 24, 1953, held in Washington, was largely a matter of hearing reports on the activity of the 23 sub-committees of the Division, a report of its Director—Dr. Paul Weiss—and a report from the War Man-Power Commission as to actions in prospect concerning plans to increase available man-power for the Armed Services and therefore a lessened number of students available for scientific training until after meeting their responsibilities to the Government. It was also pointed out that Governmental economy directives had materially cut down on the funds available for utilizing non-governmental agencies for research purposes and therefore eliminating many grants for research purposes to both institutions and individuals. One bright spot in the picture was the increased grants by Industry for research, both inside and outside of their own organizations.

The report was accepted and ordered placed on file.

4. *To the Agricultural Research Institute Organizational Meeting (B. Schwartz):* Dr. Schwartz reported that the Agricultural Research Institute was conceived and organized by scientists employed by Industry as a supporting organization for the Agricultural Board of the National Research Council with the purpose of promoting research and policies needed to insure the best long-term utilization of agricultural resources for the national welfare. Two kinds of memberships were set up: (1) Class A (dues-paying) for corporations and individuals engaged in the production, processing and/or marketing of agricultural products, etc., and (2) Class B (non dues-paying) for representatives of State and Federal Experiment Stations, Academic Institutions, Scientific Societies, etc. The report further recommends that this Society accept a Class B membership and become a part of this general movement.

The report was accepted and ordered placed on file.

5. *To the Secretaries' Conference—Member Societies of the A.I.B.S. (A. C. Walton):* The purpose of the meeting of the Secretaries of the various member Societies in attendance at Madison was to obtain suggestions for the betterment of future meetings and to explain certain of the policies of the A.I.B.S. One item of interest is that all expenses of the annual meeting and of the program are met from the registration fee and advertising, so that none of the fees paid by the individual Societies as based on their membership lists is used for the meetings. These fees go to support the general activities of the Institute as outlined in the report by Dr. Cort. No society pays more than $1,000.00, but up to that amount pays $1.00 per member unless said member belongs to two or more member Societies. In that case $0.50 is charged per member to each Society involved. Projected activities of the Placement Service and of the Service to Society Journals were explained.

The report was accepted and ordered placed on file. The Council further ordered the giving of a subscription for the Journal to the Institute to assist in preparing their Bio-Sciences News Letter to foreign countries.

6. *Report of the Delegate to the XIV International Congress of Zoologists, August 5–12, 1953, at Copenhagen, Denmark (N. R. Stoll):*

The XIV Congress will go down in the history of zoology as having accomplished more for insuring stability and universality in nomenclature than any since the Code was adopted at Berlin in 1901. This exceptional result at Copenhagen was due primarily to the technical discussions and decisions of a Colloquium on Zoological Nomenclature during a whole week preceding the Congress. The Colloquium, organized by Secretary Hemming of the I.C.Z.N., and I.T.Z.N., for interested taxonomists and members of the Commissions, held 15 half-day sessions with 35–40 regularly in attendance. A visible sign of its labors was a 48-page mimeographed report made available to the Section on Nomenclature, which, with slight emendations, became the Section's report to the plenary session of the Congress.

This adopted report of the Colloquium is to become available generally. Also to be published soon is a first draft revision of the Code under preparation by Professor Chester Bradley (Cornell), President of the International Commission, in the light of the decisions taken at Copenhagen (and Paris). Following any later changes adopted by the Commission in the light of suggestions from zoologists after studying the above, the official revised Code will be published, to formally come into force on the first day of January in the calendar year following publication. Workers in taxonomy, and editors, are asked to guide themselves accordingly during the period which must elapse before the Revised Code comes into force. Similarly the International Commission will guide itself and treat the report approved by the Congress as binding upon it during that period.

V. Old Business

1. *Permanent membership in the Institute of Agricultural Research of the National Research Council:* On recommendation of the Representative (Dr. Schwartz) to the organization meeting of the Institute, the Council voted to take up a Class B (non dues-paying) membership, and to appoint a representative to the Institute.

VI. New Business

1. *Election to Active Membership:* Nineteen applicants were elected by the Council to active membership in the Society:

Bernard, George R., Jr., 19 Irving Street, Boston 14, Massachusetts
Brun, Edwin L., Livestock Diagnostic Laboratory, Box 1951, Baton Rouge, Louisiana
Crites, John L., Room 87, Botany & Zoology Building, Ohio State University, Columbus 10, Ohio
Fitzgerald, Paul R., Zoology Dept., Utah State Agricultural College, Logan, Utah
Gastfriend, Allan, 180 N. 1st Street (Bsmt.), Tooele, Utah
Heck, Oscar B., Jr., College of Veterinary Medicine, State College of Washington, Pullman, Washington
Huff, George, Dept. Zoology, Drake University, Des Moines, Iowa
Kingscote, Anthony A., Ontario Veterinary College, Guelph, Ontario, Canada
Kraut, Nathan, Dept. Bacteriology & Public Health, Giltner Hall, Michigan State College, East Lansing, Michigan
Li, S. Y., Dept. Bacteriology & Public Health, Giltner Hall, Michigan State College, East Lansing, Michigan
Mathies, Allen W., Jr., 225 N. Wahsatch, Colorado Springs, Colorado
Radke, Myron G., 755 5th Street, North, Carrington, North Dakota
Rowan, William B., 424 E. Veterans Place, Ithaca, New York
Sanjean, John, Entomology Department, Cornell University, Ithaca, New York
Sarmiento B., Luz, Loreto 278, Lima, Peru, South America
Senger, Clyde M., 5212 S. E. Tolman, Portland 6, Oregon
Shumard, Raymond R., Dept. Zoology, North Dakota Agricultural College, Fargo, North Dakota
Teodorovic, S. D., 215 First Avenue, Highland Park, New Jersey
Zeledon, A. R., Calle 36, Avs. 4 y 6, San Jose, Costa Rica, Central America

2. *Election to Life Membership:* The Council unanimously voted to recommend to the Society the election of the following men as Life Members of the American Society of Parasitologists in recognition of their long and outstanding service in the field of Parasitology:
William A. Riley, John W. Scott, and Ernest E. Tyzzer

All of these men are charter members of the Society and have been active in its affairs. Dr. Riley served as President in 1931, has served on the Council two terms, and has served either on the Editorial Committee or the Editorial Board of the Journal of Parasitology since its founding up to the present. Dr. Scott has been active in the programs of the Society, especially in the field of Veterinary Research, and served 18 years on the Editorial Board of the Journal. Dr. Tyzzer served as President in 1934, has served one term on the Council, and one term on the Editorial Board of the Journal.

3. *Annual Meeting:* According to the policy of the Society to meet at intervals with the Society of Tropical Medicine, it has been decided to await their choice of the next meeting place at their November assembly in Louisville, so as to determine the feasibility of holding a joint session with that group next year.

If not feasible to schedule such a meeting with that group, announcement of the alternative site will be decided and an announcement will be published in an early issue of Volume 40 of the Journal. At present it seems definite that the meeting will not be with the AIBS at Gainesville, Florida, next September. The Society has received an invitation to meet with the AAAS at Berkeley in December of 1954 (see below).

4. *Nominations, Elections, and Appointments to Society Offices:*

A. *Nominations:* On the basis of 105 ballots returned in response to the Secretary's call, the following persons (in each case those who received the greatest number of votes) were nominated by the Council for the designated offices in the Society for 1953: *President-Elect,* Clay G. Huff; *Vice-President,* Donald B. McMullen; *Secretary* (2 yr. term), Arthur C. Walton; *Council Members at Large* (4 yr. term—2 to be elected), Raymond M. Cable, Aurel O. Foster; *Council Member at Large* (1 yr. term to fill vacancy), Lloyd E. Rozeboom.

B. *Elections:* The Council authorized the following elections to the designated offices: *Chairman of the Editorial Committee of the Journal of Parasitology* (5 yr. term), George R. LaRue; two *additional members of the Editorial Committee* (5 yr. terms), Clay G. Huff, Cornelius B. Philip; *Editorial Board of the Journal* (4 yr. terms—3 to be elected), W. Balamuth, W. W. Cort, H. W. Stunkard; *Custodian of the Endowment Fund* (3 yr. term), N. R. Stoll; *Custodian of Back Issues* (3 yr. term), Wilford S. Bailey.

C. *Appointments:* The Council made the following appointments: To *Council of A.A.A.S.* (2 yr. terms—2 to be appointed), W. Balamuth, G. H. Ball; to *Agricultural Research Institute Board* (3 yr. term), B. Schwartz; *Custodian of Back Issues* (to fill vacancy until January 1, 1954), W. S. Bailey.

5. *Other Business*

a. The Council decided against joining the AIBS pool to manage the finances of the Journals of the member Societies.

b. The Council decided to continue the duplication of missing back issues of the Journal, at least until complete files become available.

c. The Council re-emphasized that the preparation of Symposia is in the hands of the Vice-President whenever such Symposia or Joint Sessions with other Societies seem advisable.

d. The Council decided to continue the Demonstration Session at the Annual Meetings and urges members to make larger use of this method of presentation and thus ease the greatly crowded schedule of papers to be read.

e. The Council has now placed definite limits on the terms of office for all Officers, Members of Council, Members of the Editorial Committee, Members of the Editorial Board, Custodians, Representatives to various organizations, and to membership on Standing Committees. This in no way limits the possibility of re-election or re-appointment to the same duty for successive periods of service.

The Council voted to adjourn at 1:00 AM, September 8, 1953.

Respectfully submitted,
A. C. Walton, Secretary

AMERICAN SOCIETY OF PARASITOLOGISTS
TWENTY-EIGHTH ANNUAL GENERAL BUSINESS MEETING

SEPTEMBER 9, 1953

In the absence of the President, C. W. Philip, the general business meeting of the Society was called to order by the President-Elect, E. R. Becker, at 1:30 PM following the annual luncheon in the dining room of the Congregational Church, 1609 University Avenue, Madison, Wis. One hundred and fifteen persons were present.

1. Reports of Officers, Custodians, Committees and Society Representatives were read and approved.

2. In accord with the Constitution, the Council unanimously recommended that the Society

elect Dr. William A. Riley, Dr. John W. Scott, and Dr. Ernest E. Tyzzer to Life Membership in the Society in recognition of their outstanding services to the Society as charter members, officers, and contributors to the Society's welfare as well as in recognition of their standing in the general field of Parasitology. Election was by unanimous ballot.

3. The names of nineteen individuals elected to active membership at the Forty-Third Council Meeting were read.

4. On the basis of the report of the Society's Representative to the organizational meeting of the Agricultural Institute, the Council recommended that the Society accept a permanent Class B (non dues-paying) membership in that organization. The recommendation was accepted by unanimous vote of the Society.

5. The names of those designated to hold appointive offices and to act as Representatives of the Society or as Committee members by vote of Council were announced.

6. The Officers and Council Members of the A.S.P. as nominated at the Forty-Third Council meeting were elected to their respective positions.

7. It was announced that the place of meeting for 1954 would not be with the A.I.B.S. and that the selected site would be published in the February number of the Journal (see below).

8. Resolutions concerning the passing of Dr. H. J. Van Cleave, the resignation of Dr. Otto as Custodian of Back Issues, and on the completion of ten years of service as Editor of the Journal of Parasitology by Dr. H. W. Stunkard were read.

9. Votes of appreciation to various organizations and individuals concerned with the very successful twenty-eighth annual meeting of the Society were passed and the Secretary was instructed to convey such appreciation to the proper parties.

The Society voted to adjourn at 2:40 PM.

Respectfully submitted,
A. C. Walton, Secretary

ANNUAL MEETING, 1954

By mail vote, the Council of the American Society of Parasitologists has agreed to accept the invitation of the American Society of Tropical Medicine and Hygiene to a joint meeting to be held November 4, 5 and 6, 1954, at Memphis, Tennessee.

NOTICE

Issues of the Journal of Parasitology which are undeliverable because of changes of address are now being returned to the Treasurer at parcel post rates. As these rates have now reached 20¢ to 25¢ per returned journal, it has become necessary to pass these charges, plus forwarding rates (6¢ to 8¢), back to the addressee. In order to avoid such charges, all members contemplating changes of address are urged to notify the Treasurer of such changes sixty days prior to the time when the changes are due to become effective.

Dates for mailing of numbers of Volume 39 (1953):

No. 1, February 1953—Mailed Feb. 25, 1953
No. 2, April 1953—Mailed Mar. 30, 1953
No. 3, June 1953—Mailed May 19, 1953
No. 4, August 1953—Mailed Aug. 13, 1953
No. 5, October 1953—Mailed Oct. 16, 1953
No. 6, December 1953—Mailed Dec. 30, 1953

WANTED

Your spare copies of back numbers of the

JOURNAL OF PARASITOLOGY

Recent inroads on certain issues of the Journal have depleted the copies in stock. Duplicate or unwanted issues may be returned to the Custodian of Back Numbers where they will become available to new subscribers or members who wish to complete their files. A few complete sets are available and a large assortment of other back numbers. Prices on quotation.

Until further notice the Society will be glad to pay transportation charges on ANY back issues sent to the Custodian—

WILFORD S. BAILEY,
School of Veterinary Medicine,
Alabama Polytechnic Institute,
Auburn, Alabama

25-YEAR INDEX

(Covering volumes 1–25, 1914–1939)

of the

Journal of Parasitology

Over 9000 Entries, Subdivided

AUTHOR—SUBJECT—HOST

Included in the 3397 author entries are 1141 from abstracts of papers on the first 15 annual programs of the American Society of Parasitologists (1925–1939), and 944 from notes of Proceedings of the Helminthological Society of Washington, meetings 16–156 (Dec. 18, 1913–May 20, 1933). A special subdivision of the subject index is a 10-page list of the 684 new species of animal parasites described (including 76 new genera), made bibliographic for the whole text of the 25 volumes. The host index contains 1645 entries, 364 of them referring to man.

Students and investigators will find this perspective of a quarter century of American parasitological publication an invaluable reference work, not only for their own desks but for their institutional libraries. Copies may be obtained at $2.00 each from the Custodian, American Society of Parasitologists.

School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Alabama.